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June 1986

Proceedings of the 1984 Sugar Processing Research Conference October 16-18, 1984 New Orleans, Louisiana

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Proceedings of the 1984 Sugar Processing Research Conference October 16-18, 1984 New Orleans, Louisiana

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PREFACE

This 1984 Sugar Processing Research Conference is one of a series of conferences held in alternate years to provide a forum for exchange of information among technical leaders of the sugar industry and to report on new and noteworthy developments.

The program for this conference was arranged by Margaret A. Clarke and Mary An Godshall. The conference coordinator was Shirley T. Saucier. These proceedings were edited by Mary An Godshall.

The series Proceedings of the Sugar Processing Research Conference, of which this is the second issue, continues the Proceedings of the Technical Sessions on Cane Sugar Refining Research, which was published every other year from 1964 to 1980. For individual copies of this volume as well as back issues of the former series as long as the supply lasts, write to Sugar Processing Research, Inc., P.O. Box 19687, New Orleans, LA 70179.

Copies of this issue are also for sale at the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

ARS has no additional copies for free distribution.

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CONTENTS

The performance of ion exchange resins in decolorising carbonated liquor: An Analysis of performance data John C. Williams	1
Sensory analysis of brown sugars and its correlation with chemical measurements Mary An Godshall, Carolyn H. Vinnett, and Victor Chew	22
Recent observation on starch and sugarcane products F.W. Parrish, W.R. Goynes, E.J. Roberts, and M.A. Clarke	53
A glucan from sugarcane Earl J. Roberts, M.A. Clarke, M.A. Godshall, and F.W. Parrish	60
Performance characteristics of the CTI® apparent purity laboratory analysis system James Kysilka and Stanley E. Bichsel	72
Changes in juice composition of sugarcane as affected by post-freeze deterioration in Louisiana Benjamin L. Legendre, W. S. Charles Tsang and Margaret A. Clarke	92
Dextran - An overview of the Australian experience Philip C. Atkins and Robert J. McCowage	108
<u>Leuconostoc</u> spp. in sugarcane processing samples E.B. Lillehoj, M.A. Clarke, and W.S.C. Tsang	141
Fermentable sugars from starches and cellulosic materials Michael R. Ladisch	152
Laboratory filterability test methods Nicholas Nenadkevich	162
Sugar liquor clarification using diatomite filter aid C.W. Cain, Jr.	176
Quantification of the effects of different raw sugar impurities on filtration rates in carbonatation refineries Peter Hidi and Robert J. McCowage	186
Observations on filtration impedance in raw sugar James A. Devereux and Margaret A. Clarke	209
The chemistry of iron in the sugar refinery Richard Riffer	231

Analytical methods of measurement--a need for correspondence
Stephen A. Brooks and R.A. Melanie Pilgrim 252

Electrostatic methods to separate bone char from granular
carbon: Preliminary report
Mary An Godshall, Louis C. Weiss, M. A. Clarke,
and G. Perret 266

Color tests and other indicators of raw sugar refining
characteristics
Margaret A. Clarke, Rebeca S. Blanco, and Mary An Godshall 284

Application of GC analysis in the South African sugar industry
P.G. Morel du Boil 303

Current applications of HPLC in sugar analysis
W.S. Charles Tsang and Margaret A. Clarke 316

A Comparison of GLC and HPLC for the determination of
sugars in final molasses
T.A. Chorn and A. Hugo 331

Symposium on high performance liquid chromatography in
the sugar industry: A different aspect of high performance
liquid chromatography
Andrew M. Ho 350

Symposium on high performance liquid chromatography in
the sugar industry
Robert F. Hutton 363

THE PERFORMANCE OF ION EXCHANGE RESINS IN DECOLORISING CARBONATED LIQUOR: AN ANALYSIS OF PERFORMANCE DATA

John C. Williams

Tate & Lyle, Group R&D

INTRODUCTION

Over the years the use of ion exchange resins in the decolorisation of sugar liquors has grown. The number of papers on the subject at the 1980 SPRI Technical Session is an indication of this trend. Ion exchangers are gradually replacing the more traditional carbonaceous adsorbents, having the advantages of lower energy requirements; in-situ regeneration and, with the scope for extensive automated operation, lower manpower needs.

A batch of new ion exchange resin decolorises well in comparison with carbon-based adsorbents, but the question has always been "how long will it last". In contrast to the carbons there is no longer the gradual burning away and replacement with fresh materials.

So far, the only way to find out about the longer term aspects of resin performance is actually to use the resin for many cycles in a situation as close as possible to actual operating conditions. Many such extended trials have been carried out. Cuneen and Hawkins (1972) used a polystyrene based anion exchange resin, to decolorise carbonated liquor. The performance of a fresh resin slowly declined. Economic operation was made possible by making the maximum use of the available capacity of the partly used resin by using it to pretreat liquor fed to a further fresh resin.

Fries and Walker (1980) used a different approach. They describe a number of trials, one of which used a pair of resins treating Talofloc clarified liquors for over 100 cycles. The resin pair was an acrylic anion exchanger followed by a polystyrene, and was designed to give an extended operating lifetime. The first (acrylic) resin, being more readily regenerated, protects the second (polystyrene)

from fouling by 'strongly adsorbed colorants. The second resin, having a greater affinity for colorants, polishes the output from the first to give an acceptable product color over many cycles. The principles on which the affinity of colors for resins are based were set out by Gustafson and Lirio (1968).

The idea of complementary resins derives from early Tate & Lyle work (Cookson et al 1970) and this research was continued by examining the behavior of macroporous resins with their better regeneration behavior. This is illustrated in Figure 1 which compares the regeneration profile of a gel and a macroporous acrylic resin exhausted with the same feed material. Similar behavior was observed with polystyrene resins. The advantage of a macroporous resin is obvious; with a given level of regeneration, less color is left within the resin and so a higher proportion of the original capacity is available for use in succeeding cycles.

Thus for an extended operating lifetime a system needs to be based on macroporous resins with an acrylic anion exchanger preceeding a polystyrene. Such a combination of acrylic/polystyrene anion exchangers was tested in our extended trials; first on a laboratory scale (2 x 50 ml columns) and then in a small pilot plant (2 x 1 cu ft scale) using standard carbonatated (pressed) liquor as a feed. The results of the larger trial are described here.

The pilot scale trial formed part of an on-going project to assess the processing options available to a refinery of the future. The resin pair had to deal with all the variations in feed liquor that arose from a range of raw sugars and any changes in up-stream processing that were made to accommodate the differences between the raws. Such variations cause problems in interpreting the results of trials. In order to increase the precision of any estimates of resin lifetimes and make valid comparisons between this and other systems, we had to use statistical methods of data analysis to correct for the effects of such variations. This gave an added bonus, for the techniques used allowed us to estimate the degree to which variations in operating parameters affected performance. This, in turn, led to recommendations as to the best operating procedures and also to insights into the mechanisms of resin decolorisation.

EXPERIMENTAL

Two Pyrex cells were used (1 ft. diam. x 3 ft.) fitted with a top distributor and a Nevaclog support at the bottom. Each contained 1 cu ft of resin. They were connected in series with the acrylic resin (IRA 958 then named XE279) preceeding the polystyrene (IRA 900). Temperature was kept at 70°C - 75°C by a heated cabinet and the flow controlled at 3 (Single)

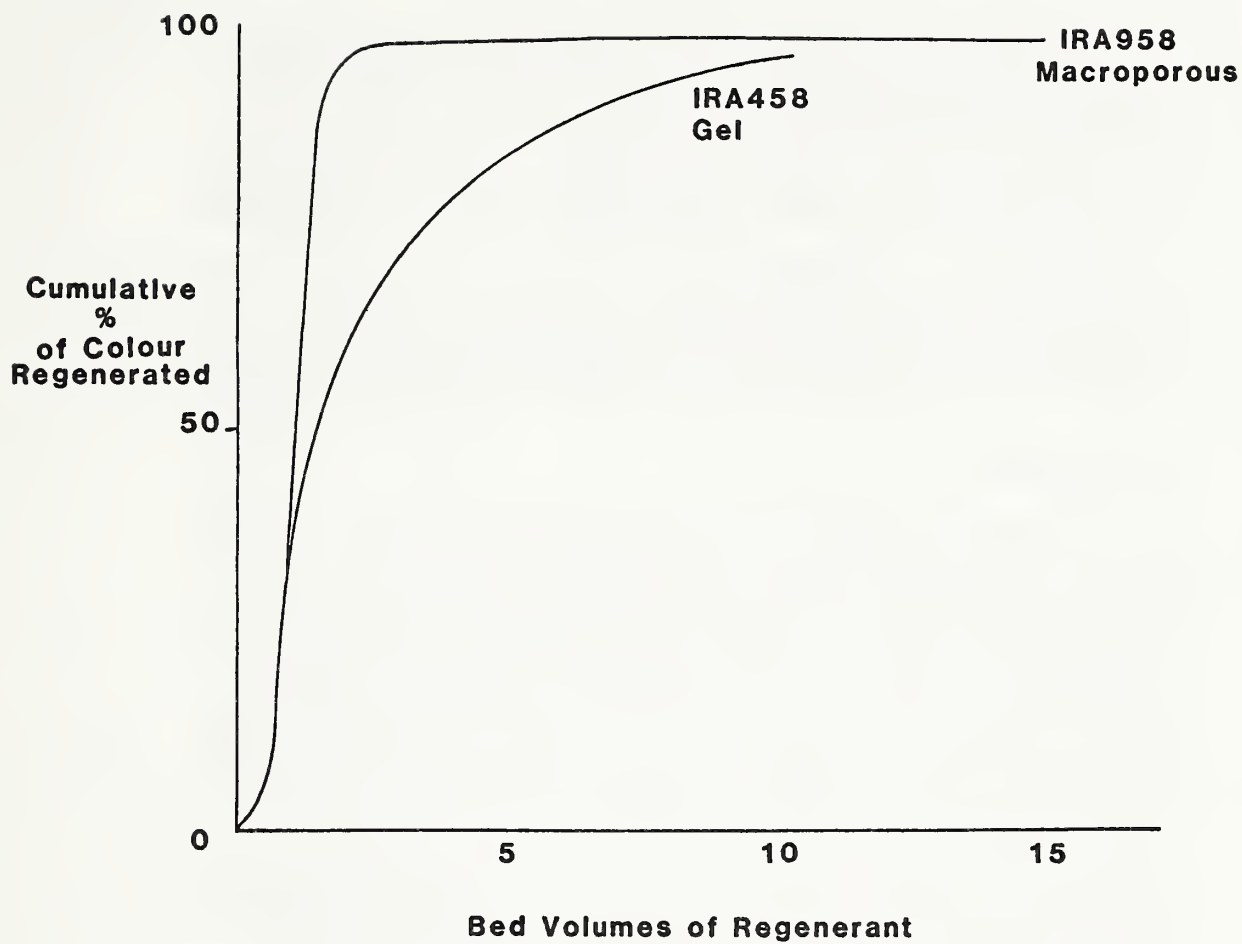


Figure 1.--Effect of resin porosity on regeneration.

Bed Volumes per hour. Each cycle lasted for 40 hours unless terminated early by process problems, or because the color loading was excessively high (exceeding 72,00 BV.ICU). After sweetening off, each resin was backwashed separately and the pair regenerated in reverse order with 4 BV of 12.5% brine. Every fifteenth cycle, the regeneration included an acid wash.

Samples of feed, acrylic product, and polystyrene product were taken every 4 hours for analysis. Colors were measured according to ICUMSA Method 4 and pH, ash, invert and Brix also determined. Table 1 contains the operating conditions for the trial.

Data Analysis

From the above it can be seen that the amount of data produced by a well managed resin trial is large, even if cycle summaries alone are considered. The calculations involved in data analysis are long and tedious (and hence error-prone) so a computer must be used. Our Digital PDP-11/10, because of other duties, had only 8K bytes of core available and had access to a 2.5 M byte disk. The data was organized into disk-based data files designed so that any data point could be recalled at will. A suite of programs was written around these files allowing tabulation and plotting of data; data transformations and multiple regression analysis which included facilities for calculating and plotting residuals and for predicting values from the derived regression equations. The greatest labor involved was loading the data into the computer.

The data analysis itself centered around multiple regression analysis. This is an extension into many dimensions of the "least squares" method of fitting a "best" straight line through a series of points in two dimensions. It is not enough to find a series of straight lines relating say, product color, to each of the variables in turn (e.g. as in Figure 2 (a and b) which shows the effects on product color of cycle number or feed color). This approach of looking at one variable at a time means that all the scatter associated with the variations in the others is included. This may give the mistaken impression that no relationship exists - or if a relationship is found it will be estimated less precisely.

TABLE 1.--Operating conditions and their effects on resin performance

Variable	Units	Mean Value and Range*	Effect of a Unit Increase in Variable on Product Color (in icu)	
			Acrylic Resin	Resin Pair
Cycle Length	BV/Cycle	120	+	+
Flow Rate	BV/hr	3	+	+
Resin Age	Cycle	up to 85	0.86 \pm .14**	0.47 \pm .09
Cycles since Acid	Cycle	up to 15	1.75 \pm .78	1.08 \pm .48
Feed Color	icu	574 (162)	0.27 \pm .05	0.15 \pm .03
Feed pH	Unit	8.64 (.36)	-	-16.5 \pm 13
Feed Ash	% on solids	0.13 (.03)	+	210 \pm 146
Feed Brix	1° Bx	64.9 (2.1)	+	+
Feed Invert	% on solids	0.13 (.06)	N	N

* Range in terms of 2 standard deviations (includes approximately 95% of all values)

** \pm 95% confidence limits

"+" or "-" No significant effect in this trial, but having a positive or negative effect in other trials.

N No effect anywhere

Additionally, there maybe a relationship between the "x" variables (albeit a coincidental one) and multiple regression is needed to sort out the effects of (for example) cycle and feed color on product color.

In effect we are asking the data "is there a real relationship between this variable and that?". The answer is given in terms of tests of statistical significance which say how real any relationship is. The actual value of the coefficients of the regression equation are compared with their variability. The answer can be expressed in such terms as "on the basis of this data you have a 95% chance of being right if you state that the coefficient of this equation lies between values 'a' and 'b'". If the limits 'a' and 'b' do not include zero then the relationship is taken to be statistically significant.

In a complex system like an ion exchange plant many variables will have their values recorded. Each of them has a potential influence on the performance of the plant. In order to find the best set of variables to represent the system a process known as backward elimination is used where all the likely variables are initially included in the multiple regression and each non-significant one eliminated in turn.

There are, of course, dangers is using multiple regression. Just because an equation says there is a relationship between two variables it does not mean that a change in one causes a change in the other. We must constantly ask ourselves "is this reasonable". A further pitfall is that if the 'x' variable changes over only a small range then any effect on the 'y' may not show up, and we are lulled into a false sense of security in thinking that 'x' has no effect on the system.

RESULTS AND DISCUSSION

Acrylic Resin

As mentioned above, a multiple regression can be regarded as a means of reducing the variability of data in order to obtain better estimates of the effects of variables. The performance of the acrylic resin (expressed in terms of product color) illustrates this. Figure 2a shows a scatter plot of product color against cycles. There is a general rise in color as the resin ages. The results are scattered over a range of approx. 0.2 a.u. Performing a simple regression of color against cycle gives the line shown in Figure 2a. The differences (called residuals) between colours predicted by this line and the actual values are shown in Figure 3 where the scatter is approximately 0.12 a.u. Take the regression a step further to include feed color and the residual band is reduced to 0.08 a.u. (Figure 4).

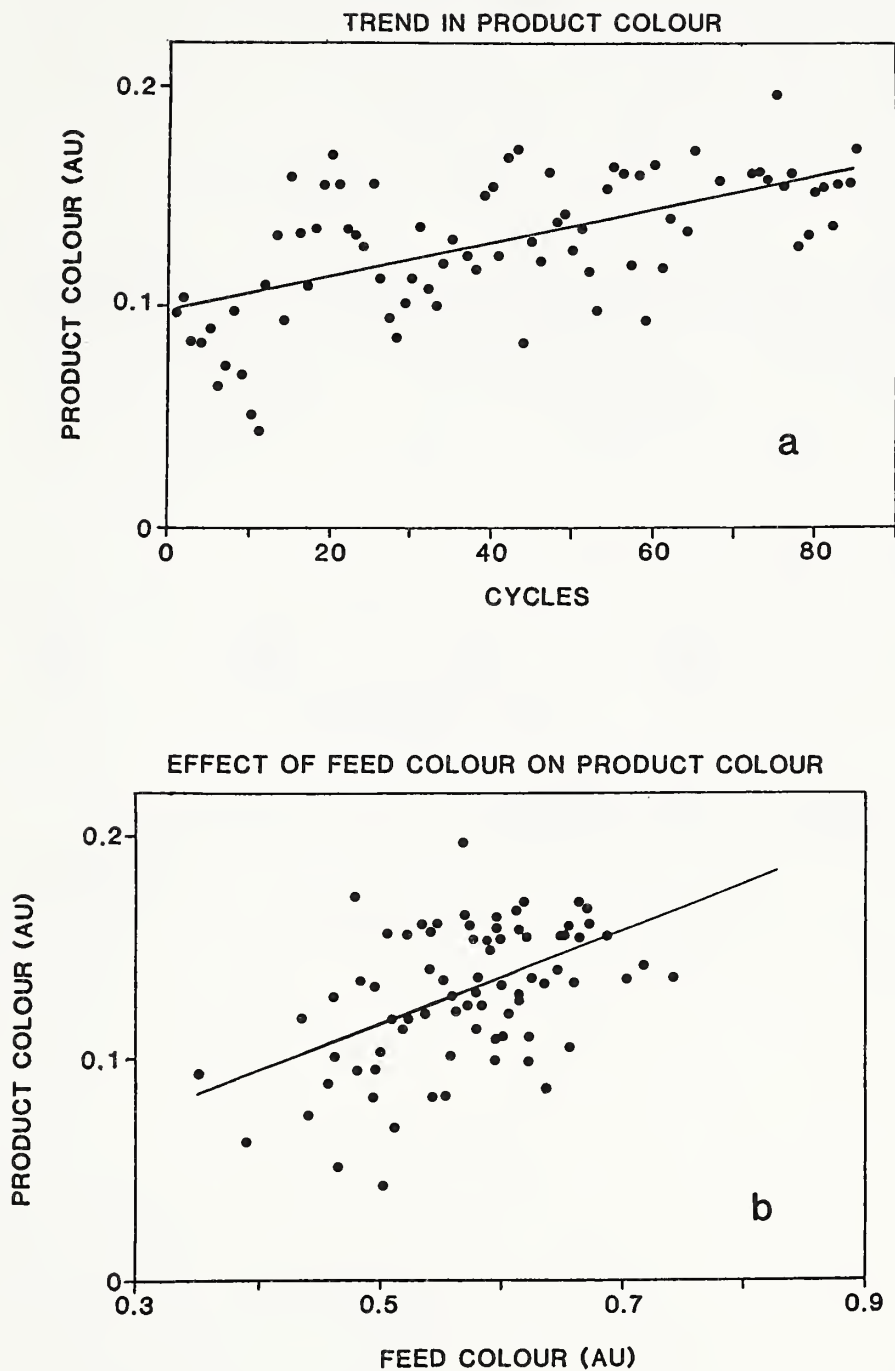


Figure 2.--Effect of (a) cycle number and (b) feed color on product color in acrylic resin.

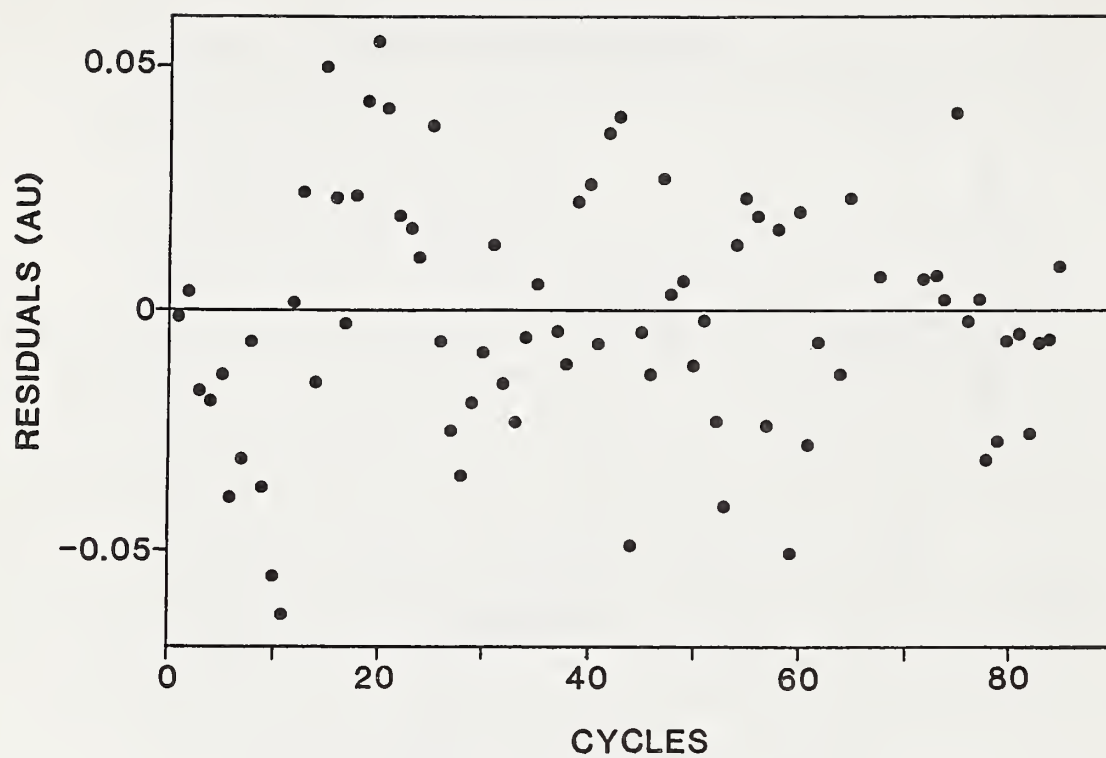


Figure 3.--Effect of cycle on product color eliminated.

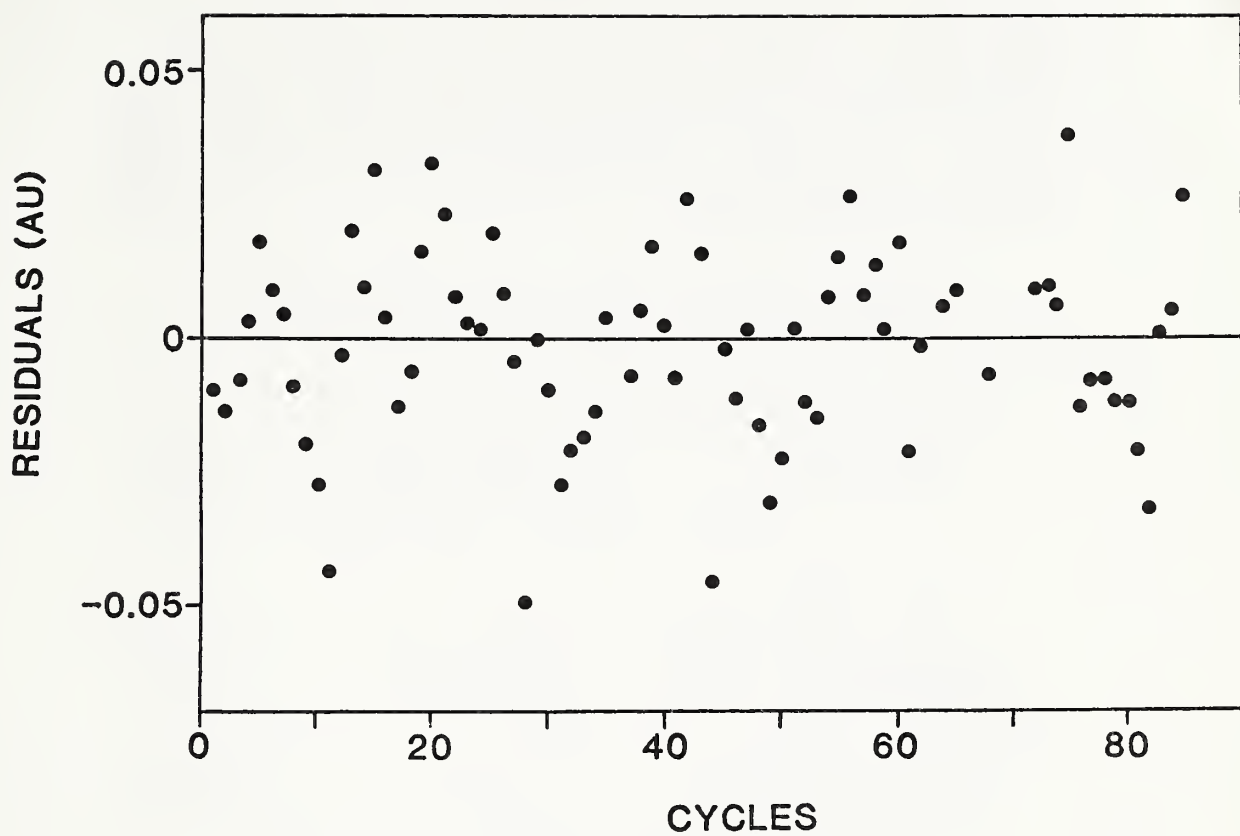


Figure 4.--Effect of cycle and feed color on product color eliminated.

There are indications of a cyclic structure in these residuals. A power spectrum of these residuals hints at an underlying frequency in the region of 15-20 cycles. As acid washes were used every 15 cycles it is reasonable to test these as a possible cause for this cyclic structure. Using the number of cycles since last acid wash (CSA) reduces the scatter a little more. The process is further shown in Table 2 as an increasing precision of the estimate of the effect of cycles on product color.

Table 2.--Effect of resin age on product color from acrylic resin : increasing precision with number of variables in regression

Regression	Estimate of Cycles Coefficient	Standard Error
Color vs Cycles	.774	.113
Color vs Cycles and Feed Color	.870	.076
Color vs Cycles, Feed Color, and CSA	.862	.068

The final equation representing the performance of the acrylic resin is:

$$\text{Product Color} = .862 \text{ cycle} + .272 \text{ color on} + .74 \text{ CSA} - 73.3 \quad (I)$$

$$\text{SEb} \quad .068*** \quad .023*** \quad .39*** \quad 14.8***$$

$$R^2 = .79$$

SEb is the standard error of the coefficients of the equation - i.e. a measure of the variability of the value.

*, **, *** indicate that the coefficients are statistically significant to 95, 99 and 99.9% levels respectively - i.e. if it is ** we have a 99% chance of being right when we say its value is not zero.

R^2 is the proportion of the total variability of the system that is explainable by this equation.

Other variables (e.g. ash, invert, pH) do not occur in this equation. This does not mean that they have no effect. It only indicates that their effect on performance is small compared with the total variation occurring.

Such equations can be used in several ways. They can predict what color product can be expected (and to what precision) with a given set of conditions (feed color, cycles run to date, cycles since last acid wash). Alternatively (as in Table 1) they can give an estimate of the effect that cycles, feed color, and acid wash have on the color of the product.

The effect of acid washing is important. Neglecting it would have caused the performance of this resin to decline three times faster. Delaney (1980) speaks of an interaction between phenolic colorants and ion exchange groups which is overcome by acid washing. An alternative effect is the removal of mechanical fouling of the resin by chalk carried over from the carbonatation process. The latter is believed to be the more important in this case because the amount of color actually eluted by an acid wash is small.

The presentation of performance as above implies that the effects of cycle, feed color and CSA are linear. The variability in the data hampers the search for curvature in the relationship. For instance, including a quadratic term (cycle squared) in the equation does not give a significant coefficient. Neither was the cycle coefficient significantly different between the first and second halves of the trial although other resin trials analysed in this manner have revealed differences implying a slight leveling off in performance decline.

An indication of non-linearity is given by the regression involving % decolorisation (in effect color off/color on):

$$\% \text{ Decolorisation} = 91.42 - .163 \text{ cycle} - 9.42 \times 10^{-3} \text{ color on} - .298 \text{ CSA} \quad (\text{II})$$

$$\text{SEb} \quad \begin{array}{lll} 2.76*** & .0126*** & 4.3 \times 10^{-3*} \\ & .073*** & \end{array}$$

$$R^2 = .74$$

Thus % decolorisation changes with feed color and by this measure the resin performs slightly less well as input color increases.

Acrylic/Polystyrene Resin Pair

Applying multiple regression to the performance of the pair of resins (IRA 958/900) gives an equation with more terms than that describing the performance of the acrylic resin alone:

$$\text{Color} = 72.8 + .468 \text{ cycle} + .148 \text{ feed color} - 16.5 \text{ pH} + 1.08 \text{ CSA} + 209.5$$

Ash

SEb	58.6(N).045***	.015***	6.5*	.24***
	73.4***			(III)

$$R^2 = .77$$

Extra parameters apparently influence the performance of the resin pair. They did not figure in the equation for the acrylic resin, but this does not necessarily mean that these did not have any effect on its performance; only that the overall variability of the system was probably such that the effects did not show up.

Comparing equation (III) and (I) the effects of cycle, color and CSA on product color from the pair appear to be half those for the acrylic alone. The effect of ash in reducing the effectiveness of the pairs' decolorisation is to be expected in terms of competition of ash for the ion exchange sites.

The effect of pH can be explained in terms of increased ionisation of colorants at a higher pH making easier their removal by an ion exchange mechanism. It is interesting to note that this pH effect is greater at lower pH's. In a series of trials where the feed averaged pH 7.2 the pH coefficient was -55 compared to -16.5 at the mean pH of the present trials of 8.6. This indicates that the mean pKa for these colorants is in the region of pH 7.

In practical terms the above equation indicates that a unit rise in pH (within the operating range of this trial) could lead to a color improvement of 16.5 ± 13 icu and every 0.01% less ash present will improve the color by 2.1 ± 1.5 icu. If the effect shown by CSA is entirely due to mechanical fouling by chalk then a completely clear feed liquor would lead to a color improvement of 8 ± 3.6 icu.

The regression equations may be used to predict the performances of a resin decolorisation system under any given set of conditions. In a working refinery, however, it is not usually practicable to control conditions closely and so variations, such as were encountered in this trial, will still affect performance. Thus a simple regression involving "cycles" alone will be the most useful in predicting performance and the likely variability of a system working within the range of variables shown in Table 1.

$$\text{Color} = 54.4 + .343 \text{ Cycle} \quad (\text{IV})$$

SEb 3.5*** .073*** $R^2 = .22$ This
 results in predicted product colors as expressed in Table 3.

Table 3.--Decolorisation performance of resin pair
 predicted by regression equations

Cycle	Predicted Mean Product Color	±95% Confidence Limits		Predicted % Decolorisation
		Mean	Individual	
1	55	7	33	91.0 (± .5)
50	72	4	32	87.3 (± .3)
100	89	9	33	83.6 (± .6)
150	106	16	36	79.8 (±1.1)

There is no guarantee that the relationships expressed in regression equations will hold outside the ranges of the available data, although the confidence limits will be calculable, if wide. Thus using these equations to extrapolate must be done with caution.

The "mean" colors and confidence limits apply to the product colors that would arise from the running of a large number of individual cell pairs under the range of operating conditions shown in Table 1. Thus over 150 cycles color will rise from 55 (± 7) to 106 (± 16) icu with a mean product of 80 (± 13). The elimination of all chalk effects would improve the mean color to 72 (± 15) icu.

The "individual" confidence limits represent the range of colors within which the product of an individual cell pair could fall, when taking into account the full variability likely in operating conditions. Over 150 cycles the product color rises from 55 (± 33) to 109 (± 36) icu. During these trials some resin was lost from each cell and thus the above predicted performances will be pessimistic.

In terms of % decolorisation of the resin pair performance maybe represented as:

$$\% = 91.04 - 0.75 \text{ Cycle} \quad (\text{V})$$

SEb .47*** .0099*** $R^2 = .42$ with
 predicted performance shown in Table 3.

It is interesting to compare the predicted performance of this 1 cu ft pair after a rudimentary correction for the resin losses, with a similar prediction for the trials employing 2 x 50 ml of the same resins which preceded this pilot scale trial. This is shown in Figure 5 with the lines and their 95% confidence regions being compared. There is apparently only a small decline in performance over this 566 fold scale-up.

Resin Cycle Profiles

In order to enhance our understanding of the processes underlying resin decolorisation in terms of the interactions of different color types with the resins, it is useful to simplify each individual cycle by expressing its profile (graph of % color leakage against proportion of cycle already run (Volume Fraction)) as a straight line (Figure 6). This line will have an intercept (I) and slope (S). "I" represents color that can never be removed by the resin under the operating conditions prevailing: either on account of being too weakly bound to the resin; being too large to enter the pores; or because the conditions of flow provide insufficient time for it to reach to adsorptive sites. "S" represents color that is initially taken up (and relatively weakly bound) by the resin, but displaced later in the cycle by more strongly adsorbed materials.

This may be illustrated by the results from the acrylic resin. Colors entering and leaving each resin were recorded 10 times in each cycle of this trial (4 hr intervals). Linear regressions were performed on each complete cycle and in the greater majority of cycles these were better than 99% significant. There were no systematic deviations in the residuals for these lines. Thus for this resin/liquor system it is reasonable to accept a linear profile.

Both intercept and slope increased with resin age and this can be expressed by equations:

$$I\% = 10.41 + .0603 \text{ Cycle} \quad (\text{VI})$$

$$SEb \quad .715*** \quad .015*** \quad R^2 = .18$$

$$S\% = 11.99 + .172 \text{ Cycle} \quad (\text{VII})$$

$$SEb = 1.28*** \quad .026*** \quad R^2 = .37$$

The increase in profile intercept may be attributed to the effects of fouling due to colorants being irreversibly adsorbed and thus blocking adsorptive sites. Past experience indicates that fouling is due to the irreversible adsorption of the highest molecular weight colorants. These can only be taken up by sites within the largest pores and once these sites are irreversibly occupied then these particular

colorants can no longer be adsorbed and will pass straight through the resin to increase the intercept color. There is evidence for this in the visible spectrum of the initial colorants eluting from a resin. The 'N-value'⁺ of these increases as the resin ages, indicating an increase in molecular weight. The N-value is an indicator of the relative molecular weight of colorants.

As a resin fouls there will be less sites available to adsorb colorants; which is the same effect as having a shorter resin column. Thus the profile slope also will increase as a resin ages. Naturally, any loss in resin that occurs will also have this effect, as will the slow degradation of the ion exchange groups that occurs with age.

Equations (VI) and (VII), although statistically significant, do not explain much of the total variability of the system. They may be combined to give:

$$\% \text{ leakage} = 10.41 + .0603 \text{ Cycle} + 11.99 \text{ vol fraction} + .172 \text{ vol fraction} \times \text{cycle}$$

(Because $\% \text{ leakage} = I + S \times \text{vol fraction}$) at any point in cycle.

Analysing the $\% \text{ leakage}$ data in terms of these 3 variables gives a very similar equation:

$$\% \text{ leakage} = 10.22 + .0635 \text{ Cycle} + 12.39 \text{ VF} + .165 \text{ Cycle} \times \text{VF} \quad (\text{VIII})$$

$$\text{SEb} \quad .66*** \quad .014*** \quad 1.07*** \quad .022***$$

$R^2 = .74$ which will account for a large amount of the system variability.

Similarly the leakage from the resin pair may be expressed as:

$$\% \text{ leakage} = 7.09 + .051 \text{ Cycle} + 4.55 \text{ VF} + 0.27 \text{ Cycle} \times \text{VF} \quad (\text{IX}) \text{ SEb}$$

$$.41*** \quad .0085*** \quad .665*** \quad .0137*$$

$$R^2 = .45$$

+ N - Value: A measure of the shape of the visible spectrum of sugar colors (Parker 1977):

$$N = 100 \quad \frac{\text{Absorbance at 520 nm}^2}{\text{Absorbance at 455 nm}}$$

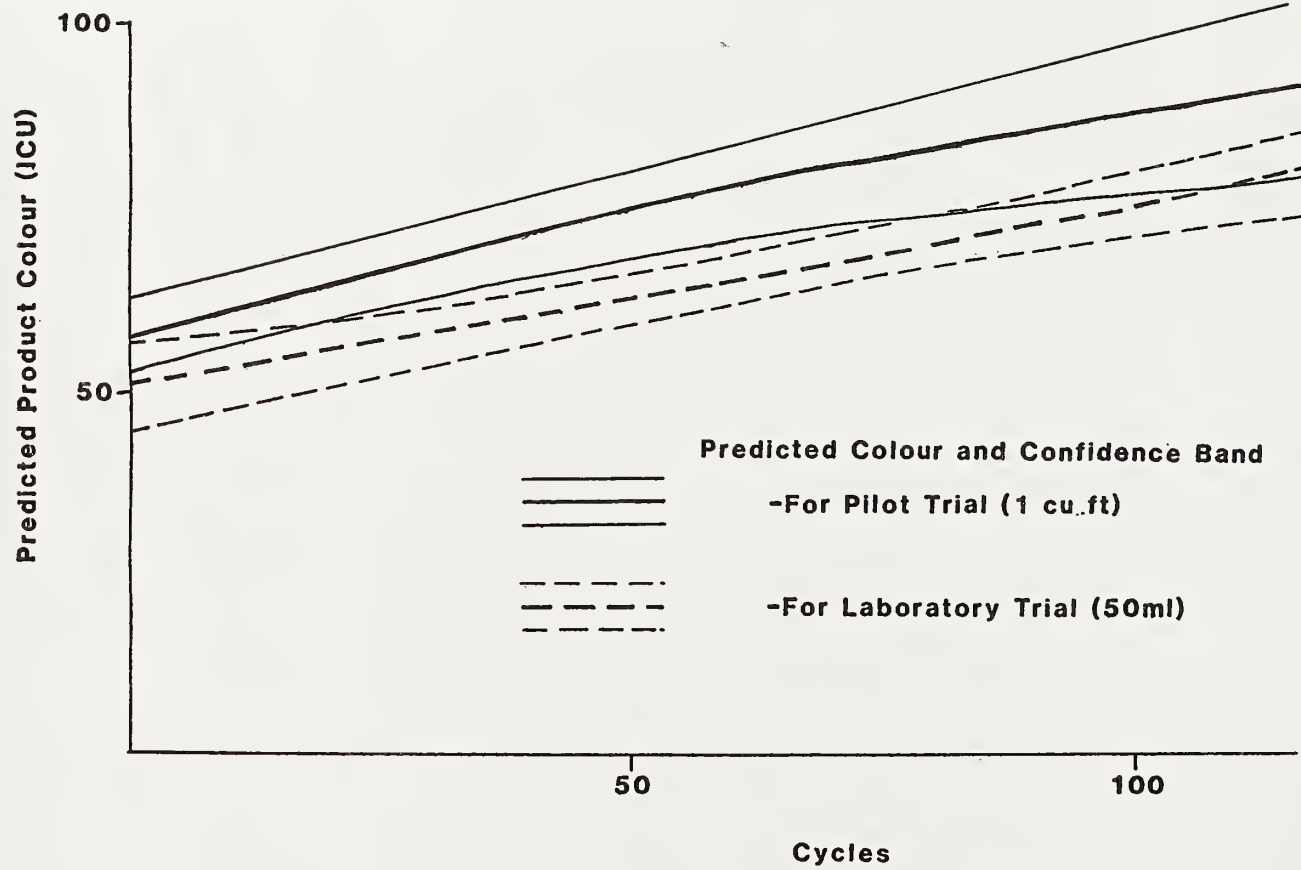


Figure 5.--Comparison of performance of laboratory and pilot trials.

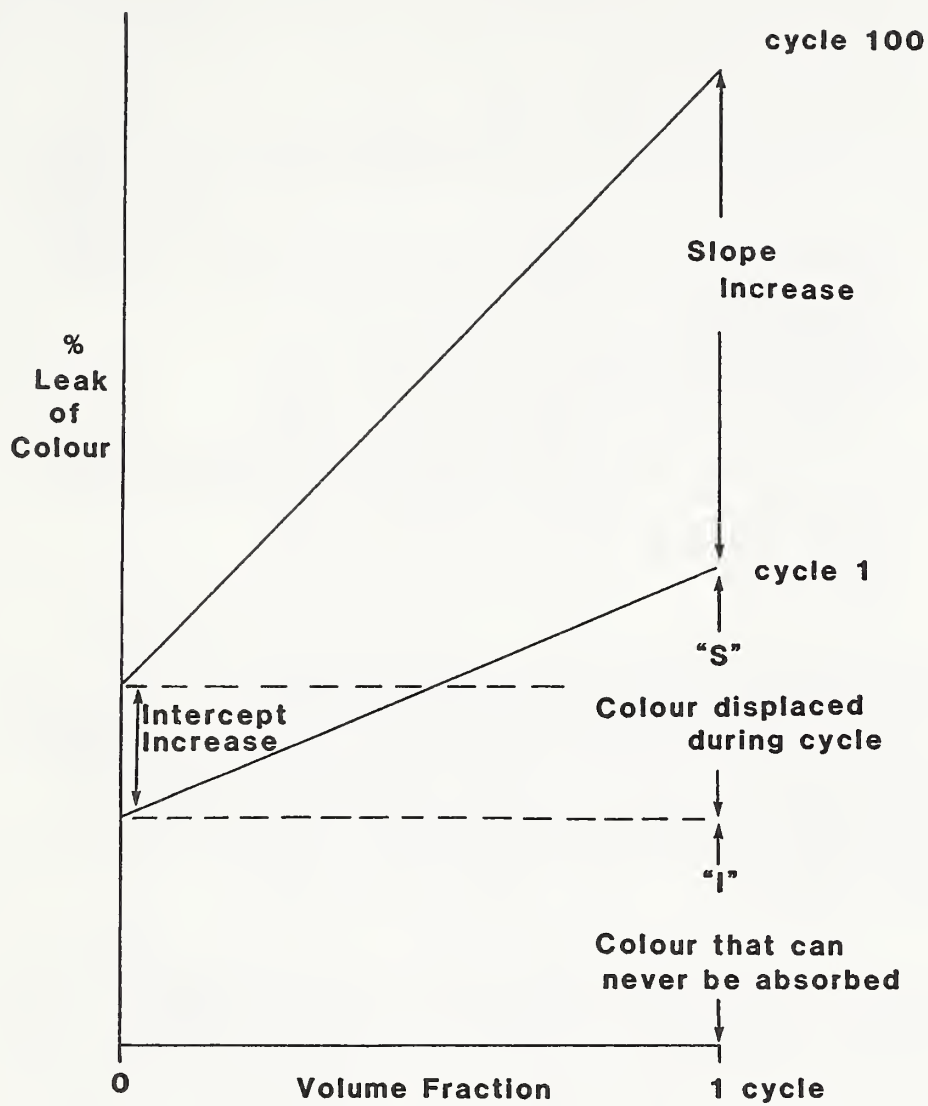


Figure 6.--Resin cycle profiles.

As may be expected the intercept and slope of the fresh pair is lower than for the acrylic alone and the performance declines more slowly.

IMPLEMENTATION ON A PLANT SCALE

The data analysis discussed indicates which factors control the performance of ion exchangers decolorising sugar liquors:

Age of Resin (in terms of cycles or cumulative color load).

Color of feed

pH of feed

Ash content of feed

Clarity of feed/frequency of acid washes

and from the analysis of other trials (not presented here):

Cycle length

Flow rate

Brix

Many of the above factors will be inter-related; either through the operation of up-stream processes (as in the case of color, pH and ash) or through the dictates of economical refinery operation (cycle length, flow rate, Brix); so the achievement of optimum resin decolorisation by their manipulation may prove very difficult. Clarity of feed, however, is controllable and should pay dividends in giving a better product.

The use of macroporous resins and acrylic/styrene pairs has gone some way towards extending the operating life of a resin system, but the analysis of cycle profiles indicates that some fouling of the resin by colorants is still occurring in the system described. One logical step is to replace the brine regenerant with alkaline brine on a regular basis. Loker (1983) found this to be an essential part of maintaining the performance of acrylic resins. It should have the effect on resin cycle profiles of lessening the increase in both intercept and slope (Figure 6) as the resin ages. However, any increase in slope due to the slow degradation of ion exchange groups will remain and may even be enhanced by the alkaline environment.

A further measure to enhance resin lifetime is suggested by the prediction from equation (II) that an acrylic resin which has served for 200 cycles will still be able to remove more than 50% of input color. If the economics of providing extra plant permit, it seems reasonable to follow Cuneen and Hawkins (1972) in employing this remaining capacity by using older acrylic resins to pre-treat liquor before it is fed to a

fresh acrylic resin.

The trials described in this paper, together with these two modifications, have formed the basis for the plans of a resin plant which will decolorise one million tons of sugar per annum at Tate & Lyle's London Refinery. The plant is due to come on stream in 1985.

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DISCUSSION

Margaret A. Clarke, SPRI: Have you looked at the types of colorants that are coming through the resin and properties, like the relationship between the size and the charge? Are they big phenolic colorants or big amines? What about the high molecular weight colorants that foul the resin? The second question is, do you have any comments on inorganic fouling with silicate or similar ions?

Williams: No comments on the question of inorganic fouling. We addressed ourselves purely to measuring colors. As regards color types, unfortunately, we haven't yet got a reliable method of distinguishing between chemical differences among colorants e.g. amine and phenolics. The colors that do get through are predictably of very low or zero charge and very low molecular weight. High molecular weight molecules can accommodate more charges, and also tend to have a greater hydrophobic interaction, thus they are very firmly held by the resin.

Robert Kunin, Consultant: You indicated you were recording data on inversion, but you didn't show any regression analysis for the inversion, and I was wondering were there any changes in invert after your acid treatment?

Williams: We weren't actually measuring inversion; but while we had the data in the computer, we looked for changes in inversion over the process. There were none.

Kunin: Even after the acid water?

Williams: They were not apparent.

Kunin: Did you use an alkaline wash after that?

Williams: On occasions it was used, but the real idea of the acid washes was to get rid of the chalk. We also didn't see any pH jumps, after these washes.

Charles Dickert, Rohm and Haas: A very interesting paper. Following up on Bob's question, was the acid regeneration a separate step after brine regeneration?

Williams: It was before brine regeneration.

Dickert: Did you try acid mixed in with brine?

Williams: No, that was never done. The brine routine was just to remove the chalk.

Stanley E. George, B.C. Sugars: You had mentioned that this ion

exchange process was used in a carbonatation refinery. Is there any problem with using phosphatated liquors?

Williams: We didn't try it on phosphatated liquors except for very limited trials, so we really couldn't compare one with the other. This refinery uses only carbonatation process, but I have heard that in Brazil they run successfully with resins and phosphatated liquors, and they haven't any problems at all.

Michael C. Bennett, Tate and Lyle: Yes, the Brazilian sugar industry does make use of the combination of phosphatation with ion exchange resins, especially in the manufacture of amorphous sugar where they are seeking a fine liquor of much lower color than the normal color found in a crystallization refinery.

SENSORY ANALYSIS OF BROWN SUGARS AND ITS CORRELATION
WITH CHEMICAL MEASUREMENTS

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INTRODUCTION

The sugar refining industry, historically, is an example of the successful blending of traditional and innovative technologies. The adoption and use of new technologies, such as ion exchange, color precipitation in clarifications, and amorphous processes, over the years, have been directed toward the dual goals of reducing costs and producing a better refined sugar, goals which have been well achieved.

The question thus arises as to what effect these changes have on other refinery products, especially brown sugar, which appeals to the consumer, not on the basis of whiteness or "pureness" but rather on the basis of flavor and appearance.

In the food industry, when a company has a successful process and product, there is understandable reluctance to change the process lest that elusive quality which distinguishes the product disappear. The sugar industry, when implementing process changes to achieve one goal (better and cheaper refined sugar) thus finds itself in conflict over the question about the resulting effect on its successful by-product sugars.

One way to approach this situation is to attempt to understand the factors that contribute to brown sugar flavor and quality.

In the present study, brown sugars from around the world, made by various processes were examined with regard to chemical composition, process variables, volatile profiles, and sensory

analysis. There were two specific objectives to the study: First, to determine if it were possible for trained flavor panelists to distinguish individual flavors within a brown sugar and to develop sensory profiles of individual brown sugars; and second, to determine if the sensory profiles could be correlated with the composition of the brown sugar or the processes used to manufacture them.

Sensory tests fall into 3 categories--difference tests, typified by the triangle test; preference tests, which measure preferences or acceptability with either ratings or hedonic scores; and descriptive analysis, which describes and measures sensory attributes, resulting in a quantitative flavor profile (Johnston, 1979). Each type of test answers a specific set of questions about a product. In this study, the flavor profile method, or quantitative descriptive analysis, was felt to be the test that would give the maximum amount of sensory information about the brown sugars, which could then be related to chemical and other instrumentally obtained data about the sugars.

Flavor profiling uses an expert flavor panel of 5 to 10 highly trained individuals to develop a quantitative flavor profile of the product. The completed profile will provide an over-all rating of the flavor (flavor score), identify individual elements of the flavor (flavor attributes or characteristics), give the intensity of each attribute (quantitation), and describe other contributions to the taste, such as odor, texture, and sound. In this study, the dimensions of odor and texture were not addressed.

The first part of the study involved the sensory analysis of brown sugars. This included several training phases and the actual sensory profiling phase. The training phases required training a panel of at least 10 people to recognize individual flavors in brown sugars. They were then required to identify individual flavors and to rate their intensity. A common vocabulary had to be developed and a set of flavor standards had to be chosen for the training modes. The panelists were then asked to do a sensory profile of the individual brown sugars using the flavors and terminology developed in the training phases.

Previous work in this laboratory (Godshall, 1981) had led to the identification of several important brown sugar flavor constituents. This knowledge served as the starting point for choosing flavors to consider in a descriptive sensory analysis of brown sugars.

The second part of the study involved the chemical analysis of the brown sugars. A "profile" of objective parameters was developed for each sugar. Statistical analysis was used to determine if there were correlations of the flavor scores and other sensory attributes with compositional data and process parameters.

MATERIALS AND METHODS

Sugars Used. Twenty-seven sugars were subjected to sensory and chemical evaluation. These were produced by American, Canadian and offshore refineries and included turbinado, light brown, dark brown, and amorphous sugars. The light and dark brown boiled sugars all came from bone char refineries. A description of the sugars is contained in Table 1.

Preparation of Candies. The sugars were prepared into a fondant-type candy using methods similar to those recommended by Swaine (1958) and Christianson and Anhaizer (1980). In order to train a panel to recognize and identify flavors that may be in brown sugars, a series of candies were made up from refined sugar with added color and one added flavor. The color was added to simulate brown sugar color without adding any brown sugar flavor. The flavors were chosen on the basis of previous research on flavor constituents (Godshall, 1981) and on discussions with producers. Concentration ranges were chosen from usage levels in foods (Furia and Bellanca, 1975) and on threshold data.

The recipe for the flavor-modified candies was the following: 400 g refined sugar was placed into a 2-qt aluminum pan. To this was added 1 g Williamson Caramel Color No. 105 (single strength acid proof) and 110 ml double-deionized water. The contents of the pan were heated to 115.5° C without stirring and removed from the heat. The pan was allowed to cool, uncovered, to 85-90° C. At this time, 200 ul glycerine was added (to give a smooth texture) and the desired flavor. Water-soluble flavors were added in a small volume of water (1-3 ml) with a pipet. Sodium chloride and caffeine were added prior to cooking. Difficultly soluble compounds such as maltol, furaneol and naringen were introduced in ethanol solutions (not to exceed 200 ul) after cooking. The cooked mass was stirred with a wooden spoon until it just started to thicken, and was poured onto a cool, smooth surface, spread to about 0.5 cm thickness and allowed to cool. When cooled, it was cut into one inch square pieces and frozen immediately in sealed mason jars. To taste, the frozen candies were removed from the jar and placed as a single layer onto paper toweling until they had reached room temperature.

To make a batch of candy from the brown sugars, the above procedure was followed using only the brown sugar and water. No color, glycerine, or flavor was added. For dark brown sugars, 25% refined sugar was substituted (i.e., 300 g dark brown sugar and 100 g white sugar per batch) to allow crystallization.

Training the Panel The first phase of the training process consisted of screening volunteers for their sensitivity to and ability to identify the four basic tastes--sweet, sour, bitter and salty. Volunteers who were able to correctly identify the lowest

Table 1.--Description of brown sugars used for flavor evaluation

Sugar*	Process Code**	Description and Origin
A-1	LB	Light brown, boiled, U.S.A.
A-2	DB	Dark brown, boiled, U.S.A.
B-1	LC	Light brown, coated, U.S.A.
B-2	DC	Dark brown, coated, U.S.A.
C-1	LB	Light brown, boiled, U.S.A.
C-2	DB	Dark brown, boiled, U.S.A.
D-1	LB	Light brown, boiled, U.S.A.
D-2	DB	Dark brown, boiled, U.S.A.
E-1	LB	Light brown, boiled, U.S.A.
E-2	DB	Dark brown, boiled, U.S.A.
F-1	LB	Light brown, boiled, Canada
F-2	DB	Dark brown, boiled, Canada
G	LB	Light brown, boiled, offshore
H-1	LB	Light brown, boiled, Canada
H-2	DB	Dark brown, boiled, Canada
I-1	LC	Light brown, coated, U.S.A.
I-2	DC	Dark brown, coated, U.S.A.
J-1	LC	Light brown, coated, Canada
J-2	DC	Dark brown, coated, Canada
K	T	Turbinado, Louisiana washed raw
L	T	Turbinado, Colombia washed raw
M	T	Turbinado, "Sugar-in-the-Raw"
N-1	LC	Light brown, coated, offshore
N-2	LC	Light brown, coated with defecated molasses, large crystal, "demerara", offshore
N-3	LA	Amorphous, high temperature process, offshore
N-4	DA	Amorphous, high temperature process, offshore, not a commercial product
N-5	LA	Amorphous, high temperature process, offshore, not a commercial product

* Sugars beginning with the same letter are from the same refinery or manufacturer.

** LB = light boiled, LC = light coated, DB = dark boiled, DC = dark coated, T = turbinado, LA = light amorphous, DA = dark amorphous.

concentration of the taste were selected for the next phase of the training. About 10% of the volunteers failed to meet this criterion.

The second phase of panel selection consisted of presenting volunteers with solutions containing varied concentrations of sucrose (3-12%). They were asked to rate the intensity of sweetness. Those prospects who were able to rate the intensity of sweetness within established ranges for each solution were selected for panel training. During this training period, panelists were repeatedly presented with sucrose solutions until a decline in mean intensity values was clearly demonstrated. This phase helped to desensitize the panelists to sweet taste.

The third phase of training involved presenting modified samples of fondants to candidates. The fondants were presented in a series of triangle tests in which the difference between the control (no added flavor) and a modified fondant (with added flavor) was established. The purpose of this phase was twofold: to determine a recognition level needed for training and to develop a common vocabulary of descriptors associated with the marker at the recognition level. The samples were also presented to the panel in a round-table format with open discussion of the tastes/flavors perceived. After the discussions, they were again presented training samples in testing booths with red light (to prevent influence by color differences, if any). At this time it was determined what percentage of the panelists could correctly identify the flavor at the different concentrations. Figure 1 shows the instruction and response sheet used for this part of the study. The flavors used to train the panelists, along with the training levels, and the recognition levels, are listed in Table 2.

The final training was conducted with selected sugars using procedures established during training. At this time, the final vocabulary for the quantitative descriptive analysis of flavor was developed.

Sensory Evaluation of Samples. Samples were presented in individual booths lighted with red light to minimize color differences. The booths contained a set of instructions, a response form, pencil, expectorant cup, and a glass of water. Three samples, in covered petri dishes, were presented on each occasion. Experimental designs for presentation of the samples were chosen from Cochran and Cox (1957). The plans used were chosen to allow a balanced presentation of 3 samples at a time, to avoid fatigue. The results were combined for a single statistical analysis.

Seventeen of the samples were subjected to two taste sessions while ten were profiled by a single session. Single session sugars were evaluated by 6 to 8 panel members, and two-session sugars were evaluated by a total of 10 to 14 panel members.

(Row #)

SENSORY ANALYSIS

NAME _____

DATE _____

You will receive three (3) samples to evaluate. Two of these three samples are identical, the third is odd or is different.

Taste the samples in the order in which they have been presented. Rinse the mouth thoroughly. Taste small bites of all of each of the samples. Allow the sample to melt in your mouth. Savor the sensations. Report your findings in each of the sections on this response form.

PLEASE CHECK THE ODD SAMPLE OR THE SAMPLE WHICH IS DIFFERENT.

Sample Codes

Check the Odd Sample

INDICATE THE DEGREE OF DIFFERENCE BETWEEN THE DUPLICATE SAMPLES AND THE ODD SAMPLE

None _____

Slight _____

Moderate _____

Extreme _____

Describe the taste and intensity of taste other than sweetness detectable in the duplicate samples and the odd sample

RESULTS

Character Notes	Duplicate Samples	Odd Sample
Caramel		
Buttery, Butterscotch		
Molasses		
Licorice		
Sulfur		
Charred, Burnt		
Sour		
Bitter		
Dry, Astringent,		
Metallic		
Green, Grassy		

92-047

Figure 1.--Instruction and response sheet for triangle test used to train sensory panel to distinguish individual flavors in brown sugars.

Table 2.--Standards used to train panelists to identify brown sugar flavors

Ingredient (Taste)	Training Levels,ppm	Identification Level, ppm	%Correct Responses***
Molasses (Molasses)	0.2%, 2.7%	2.7%	88-95
Fe ₂ SO ₄ (Metallic)	2.8, 5.6, 7.8, 17.6	17.6	65
NaCl (Salty)	100, 1000, 3750	3750	30
Furaneol (Caramel)	37.5, 56.3	56.3	52
Maple Lactone (Caramel)	39, 50	50	63
Ethyl Maltol (Caramel)	43.8, 50	50	24
Tannic Acid (Astringent)	7, 56, 111, 200	200	35
Diacetyl (Butterscotch)	12.5, 25	12.5	65-90
Caffeine* (Bitter)	200, 500, 780, 1500	1500	53
Naringen (Bitter)	200, 500	500	82
Acetic Acid (Acetic)	250, 500, 1000	1000	33
"Licorice"** brown sugar (Licorice)	100%	100%	63
Cis-3-Hexenol (Green)	0.2, 1.0, 2.0	0.2	80

*Caffeine was not a successful bitter standard; naringen was later substituted.

** Provided by a sponsoring refinery.

***Percent population expected to recognize the taste, at the identification level, after training.

A copy of the response form is shown in Figure 2. A copy of the instructions is given in Figure 3. An intensity scale ranging from 1 (not detectable) to 9 (extremely strong) was provided for rating the intensity of perceived tastes. The scale for the flavor score ranged from 1 (extremely poor) to 9 (excellent). Both scales were presented at every session.

Chemical Analysis of the Sugars. Profiles of the neutral volatiles in brown sugars were obtained with a 5750 Hewlett Packard Gas Chromatograph fitted with a Dupuy inlet (Godshall et al, 1980). Three grams of brown sugar was evenly tamped into a glass sample holder and heated inside the inlet at 135° C for 10 min. The vapors were passed through a cool condenser containing Na₂SO₄-impregnated glass wool to trap acidic volatiles. Volatiles were separated on a 6-ft nickel column, 1/8" diameter, packed with Tenax-GC coated with 7% poly-MPE. The temperature was programmed to rise to 80° C during a 4 min hold, then to rise 4° C/min to 200° C and hold for 6 min. After the chromatogram of the neutral volatiles was obtained, the trapped acidic volatiles were desorbed from the condenser at 200° C for 10 min and programmed in the same manner.

Acetic acid was analyzed in a similar manner using the Dupuy inlet with the condenser maintained at about 200° C so as not to trap any acids (Godshall, 1984). A smaller sample size of 100-300 mg was used.

Titrateable acidity was determined using normal NaOH to pH 7 and reported as mEq NaOH/g sugar x 1000.

Color was determined using ICUMSA method No. 4 (Schneider, 1979), to give the 420 color.

Phenol was determined using the Folin-Ciocalteu reagent and reported as phenol absorbancy units (Godshall and Roberts, 1983).

Iron was determined using bathophenanthroline according to the method of Lee and Clydesdale (1979).

Chloride ion was determined with a chloride-specific electrode using KCl as the standard, and is reported as ppm chloride.

Ash was determined as the conductivity ash using the ICUMSA method for high-ash samples (Schneider, 1979).

Amino nitrogen was determined by the method of Moore and Stein (1954) using ninhydrin and hydrindantin and reported as ppm leucine equivalents.

Glucose, fructose, and sucrose were determined by gas chromatography. The reducing sugars were converted to the oxime

(Row #)

SENSORY ANALYSIS

Name _____

Date _____

TASTING INSTRUCTIONS ARE LOCATED IN EACH TASTE TESTING BOOTH.

RESPONSE RESULT REPORT FORM

<u>CHARACTER NOTES</u>	<u>(Attribute)</u>		
	<u>Sample #1</u>	<u>Sample #2</u>	<u>Sample #3</u>
	<u>(Code)</u>		
<u>Caramel</u>			
<u>Fruity</u>			
<u>BUTTER, BUTTERSCOTCH</u>			
<u>NUTTY</u>			
<u>Molasses</u>			
<u>Licorice</u>			
<u>Sulfur</u>			
<u>Charred, Burnt</u>			
<u>Bitter</u>			
<u>Sour</u>			
<u>Dry, Astringent, Puckery</u>			
<u>Metallic</u>			
<u>Green, Grossy</u>			
<u>Sweet</u>			
<u>OTHER</u>			
<u>Flavor Score</u>			
<u>Comment</u>			82-CHV

Figure 2.--Response form used in sensory profiling of brown sugars.

INSTRUCTIONS FOR SENSORY ANALYSIS OF BROWN SUGAR

You will receive only three samples to evaluate. Examine these samples for those properties which appear on this response form.. Report all other tastes perceived under either COMMENT or OTHER section.

GENERAL INSTRUCTIONS:

CAUTION: TASTE ALL OF EACH SAMPLE, BUT TASTE SMALL AMOUNTS OF EACH AT A TIME:
TASTE THE SAMPLES FROM LEFT TO RIGHT OF THE TRAY.

DETAILED INSTRUCTIONS:

1. Rinse the mouth well.
2. Taste the samples in the order presented.
3. Taste sample #1. Allow the sample to melt in your mouth. Savor the sensation.
4. Examine the product for the sensory characteristics below. Report any other character notes perceived.
5. Rate the intensity of each character note's presence.
6. Rinse the mouth well and pause for a few seconds.

REPEAT PROCEDURES FOR EACH OF THE TWO (2) REMAINING SAMPLES.

Figure 3.--Instructions presented to panel during sensory profiling of brown sugars. Panels were also presented with the intensity rating scale.

using a buffered oxime reagent (Schaffler and Morel du Boil, 1981). Sugars were dissolved in water and a small aliquot (5 ul) was treated with the oxime reagent, heated 30 min at 80° C to oximate, and silylated with equal portions of TSIM and TMCS. In the presence of water, these reagents cause a two-phase system to develop. The bottom phase solidifies, concentrating the sugars in the upper phase. Internal standards consisted of quinic acid for the reducing sugars and α,α -trehalose for the sucrose. The sugars were separated with temperature programming on a Hewlett Packard 5880 Gas Chromatograph fitted with a 12 m fused silica capillary column coated with OV-101.

Statistical Analysis. Statistical analysis was conducted using SAS programs. Analysis of variance was used to test the significance of the results, and multiple linear regression and stepwise regression were used to determine correlations.

RESULTS AND DISCUSSION

Training Results and Flavor Interactions. When the flavor-modified candies were presented to the panel for the first time, the results demonstrated the panelists' difficulty with identifying flavors in the presence of sucrose. This was partially attributed to their unfamiliarity with the flavors, especially in the presence of sucrose, which would cause some modification of the usual taste as it is perceived in water. However, the results also indicated that the concentrations were, in general, too low to provide a clearly identifiable taste. Exceptions were molasses flavor, correctly identified by 55% of the panelists at the 0.2% level; butterscotch, correctly identified by 47% at the 12.5 ppm level; cis-3-hexenol, identified correctly by 56% at the 1.0 ppm level; and Fe_2SO_4 , correctly identified as metallic by 30% at the 7.8 ppm level on the first presentation. The percent of correct responses for the other flavors was around 15-20% for the highest concentration.

Nevertheless, a descriptor was usually applied to the modified candies, which indicated the possibility that flavor interactions with sucrose were occurring or subthreshold effects were operating. The panelists often made the comment that something was "different" from the control but they could not quite tell what it was. Caffeine appeared to be particularly capable of eliciting numerous incorrect descriptive terms. Among the descriptors used for caffeine were buttery, molasses, astringent, metallic, coffee and charred. Only 9% of panelists correctly identified the flavor as bitter when 782 ppm was presented the first time. It was decided that caffeine was not a successful bitter standard for use in sugar candies because of these effects. It is to be noted that subthreshold effects of this type have been noted in coffee (McCamey and Thorpe, 1984).

Other compounds tested for their ability to induce a bitter flavor to sucrose included KCl, CaCl₂, and naringen. KCl, up to 1000 ppm, did not impart a bitter flavor. CaCl₂, while imparting bitter flavor at 500 ppm, prevented crystallization. Naringen was finally chosen as a bitter standard because it imparted a bitter flavor at 500 ppm, and was structurally more similar to the flavonoid type compounds found in sugar colorant than caffeine. However, 200 ppm naringen added to sugar candies imparted a slightly pleasant caramel taste, again indicating the possibility of flavor interaction with sucrose or a subthreshold effect. Naringen did produce a moderate bitter flavor at the 500 ppm level.

Another difficult flavor to reproduce was the charred flavor which is typical of bone-char liquors. This can be quite unpleasant if it is very strong. Several possible sources of charred flavor were explored, but a satisfactory char-flavored candy was not obtained until after the training had been completed and sensory profiling was underway. Addition of sugar caramelized almost to the burnt stage gave no char flavor, but rather a mildly caramel taste. A boiling water extract of service char imparted a char odor but not a char flavor. The use of the corn protein, zein, in a slightly scorched taste was found to simulate the char flavor successfully. The idea for this came from the observation that methanolic extracts of service char had an odor reminiscent of burnt hair and gelatin, i.e, protein.

During the training period, the word "char" was equated with "burnt". By virtue of common experience and shared discussions, the panel were able to agree on its meaning and intensity, and thus the term was included in the set of descriptors used to profile the sugars.

Several compounds were tested for use as a caramel standard, including maltol, ethyl maltol, maple lactone, and furaneol. While caramel was used as a descriptor in all samples, it was the majority descriptor only in the case of furaneol (37.5 ppm). Nevertheless, only 19% of panelists described it as having caramel flavor on the first occasion. This improved to 53% with training.

The licorice flavor was suggested as important by a sponsoring refinery, who provided a sugar with moderate licorice flavor. A sugar described as having licorice flavor results from syrups boiled at low temperature and pressure for a long time (10 to 12 hours at 26 inches of vacuum). The panelists learned to identify the flavor without difficulty.

The panel's ability to identify tastes improved markedly with training. Table 2 shows the concentration level at which at least 50% of the panelists could identify the flavor, with the exception of salty (30% recognition at highest concentration level used, 3750 ppm), astringent (35%), and acetic (33%). The identification levels were rated of moderate intensity by the panel.

As a result of the training and preliminary tasting of brown sugars, 13 flavor attributes (shown in Figure 2) were chosen for the sensory analysis of the brown sugars. The descriptors fruity and nutty were not originally included but were suggested by the panel during training. It was noted that 1000 ppm acetic acid in fondants elicited the descriptor "fruity" as the majority descriptor. Since acetic acid occurs in concentrations ranging from about 30 ppm (turbinado sugars) to 1000 ppm, with an average around 350 ppm for soft sugars, this descriptor may be indicative of acetic acid content.

In summary, the training results showed that sugar masks other flavors and also modifies them, making identification of individual flavors difficult. Rather than eliciting the expected descriptor, fairly high concentrations of added flavors (i.e., 1000 ppm NaCl, 500 ppm caffeine, 500 ppm acetic acid) elicited descriptors such as molasses, caramel, and butterscotch. With training, however, sensitive individuals could be trained to distinguish individual flavors and measure their intensity.

Sensory Analysis

Flavor Characteristics of the Sugars. The combined sensory evaluation of the sugars gave 14 separate measures for each of the 27 sugars. These included the intensity ratings for the 13 flavor attributes and the over-all flavor score for each sugar. The flavor score was considered to be a measure of the quality of the sugar.

The results showed that the perceived intensity of the flavor attributes was low. This is not surprising when the intensely sweet sucrose matrix is considered. The highest intensities recorded were for char (maximum intensity was 5.57), molasses (maximum was 5.86) and licorice (maximum was 3.83). Table 3 shows the range of intensities obtained for each attribute, the percentage of samples (out of 27) in which the flavor was detected, and the results of the analysis of variance for significant differences among samples for each flavor.

Analysis of variance showed that there were significant differences among the samples with respect to molasses, licorice, char, and green flavors, as well as flavor scores at the 5% significance level ($P=0.05$). The other 9 flavor attributes (caramel, fruity, butterscotch, nutty, sulfur, bitter, acetic, astringent, and metallic) were too low to contribute significantly to the flavor profile. However, when only the two-session sugars ($n=17$) were subjected to analysis of variance, fruity flavor ($P=0.04$) and sulfur flavor ($P=0.0005$) were also found to be significantly different among samples.

Table 3.--Maximum and minimum values of intensities reported for flavors in brown sugars

Flavor	Max and min values	Percent sugars in which flavor was noted	PR>F*
Molasses	5.86 - 1.83	100	.0001
Char	5.57 - 1.10	100	.0002
Licorice	3.83 - 1.00	96.3	.0026
Butterscotch	3.19 - 1.09	100	.12
Caramel	3.09 - 1.00	96.3	.27
Sulfur	2.33 - 1.00	85.2	.15
Bitter	2.25 - 1.00	59.3	.41
Fruity	2.18 - 1.00	92.6	.12
Green	2.18 - 1.00	22.2	.0001
Nutty	2.08 - 1.00	66.7	.20
Astringent	1.93 - 1.00	74.1	.18
Metallic	1.67 - 1.00	81.5	.81
Acetic	1.38 - 1.00	29.6	.37

* PR>F = .05 demonstrated that a significant difference occurred among the samples for that attribute at the 5% level or better.

Twenty-seven sugars were evaluated for the intensity of each flavor attribute listed. 1.00 = not detected; 2.00 = barely detected; 3.00 = slightly detected; 4.00 = clearly detected; 5.00 = moderate; 6.00 = slightly strong.

A fruity flavor was not originally considered to be a brown sugar flavor attribute but was suggested by the panelists. It was detected in low levels in most of the sugars (all but 2). We found that ethyl acetate extracted a strongly fruit-flavored essence from brown sugars, and it is possible that some individuals are more sensitive to this than others. A fruity flavor may also be indicative of acetic acid content since acetic acid elicited a fruity flavor in the training samples. It is also instructive to note that while most of the samples contained no perceived green flavor (21 of the 27), this attribute was significant in the analysis of variance. The reason for this was the turbinado sugars (sugars K, L, and M). These sugars, which are more similar to raw sugar than the other sugars, had the three highest green flavor scores. Thus, a green flavor would contribute to a brown sugar sensory profile only if it were a turbinado sugar or in some way similar to raw sugar. Green flavor was correlated to fruity ($R=+0.55$, $P=0.001$ for 27 samples), indicating that if green flavor was present, a higher fruity flavor would also be noted. Nevertheless, a fruity flavor was not correlated to the flavor score ($R=-0.096$) nor to acetic acid content ($R=-0.099$).

The three most intensely char flavored sugars were one of the amorphous sugars (N-3) and 2 boiled sugars from bone char refineries (C-1, G). The three lowest char flavored sugars were a turbinado (Sugar in the Raw) and 2 light coated sugars (I-1, N-2).

Correlation of Flavor Attributes to Flavor Score. It was of interest to determine if any of the flavor characteristics (attributes) were correlated to the flavor score. This information could be used to decide which descriptors were the most useful in quality determinations.

Regression analysis of the flavor score against each of the flavor attributes showed that flavor score had significant negative correlation to licorice flavor and char flavor ($R=-0.517$ for both, significant at $P=0.01$). Multiple linear regression and stepwise regression were used to determine which combination of flavor characteristics correlated best to the flavor score. The best predictive model with 5 flavors ($R^2=0.6215$) included char ($P=0.0049$), licorice ($P=0.0095$), green ($P=0.0135$), molasses ($P=0.10$), and caramel ($P=0.17$) flavors. In this model, molasses and caramel were not significant at the 0.05 level. Quadratic regression showed that the relationship of molasses flavor to flavor score was significant for the squared term ($P=0.005$), much more so than for the linear term ($P=0.66$). Thus, the relationship of molasses flavor to brown sugar flavor is complex. This type of relationship is often seen in flavor research (Figure 4). This means that a too-low or too-high flavor intensity produces a negative rating, but some optimum intermediate intensity is desirable.

In summary, only char, licorice, molasses and green flavor correlated significantly with the flavor score. Since these four attributes also differed significantly among samples, it can be concluded that the panelists were using these four flavors predominantly to determine the flavor score. It was found also that char flavor and licorice flavor had a positive correlation to each other ($R=0.44$, $P=0.05$), indicating that these attributes, while different, did tend to track together.

The least significant attributes were butterscotch, sulfur, astringent, metallic, nutty, acetic, fruity, and bitter. All of these flavors do exist in brown sugars and could significantly alter their quality if present in moderate levels. However, in the samples tested, their levels were quite low and thus did not contribute to the over-all flavor score as much as did char, licorice, molasses, and green flavors. In future sensory profiling of brown sugar, it would be possible to cut down on the number of attributes to be rated by a panel, with the least significant flavors being described in the "comment" or "other" section of the response form, only if they were perceived.

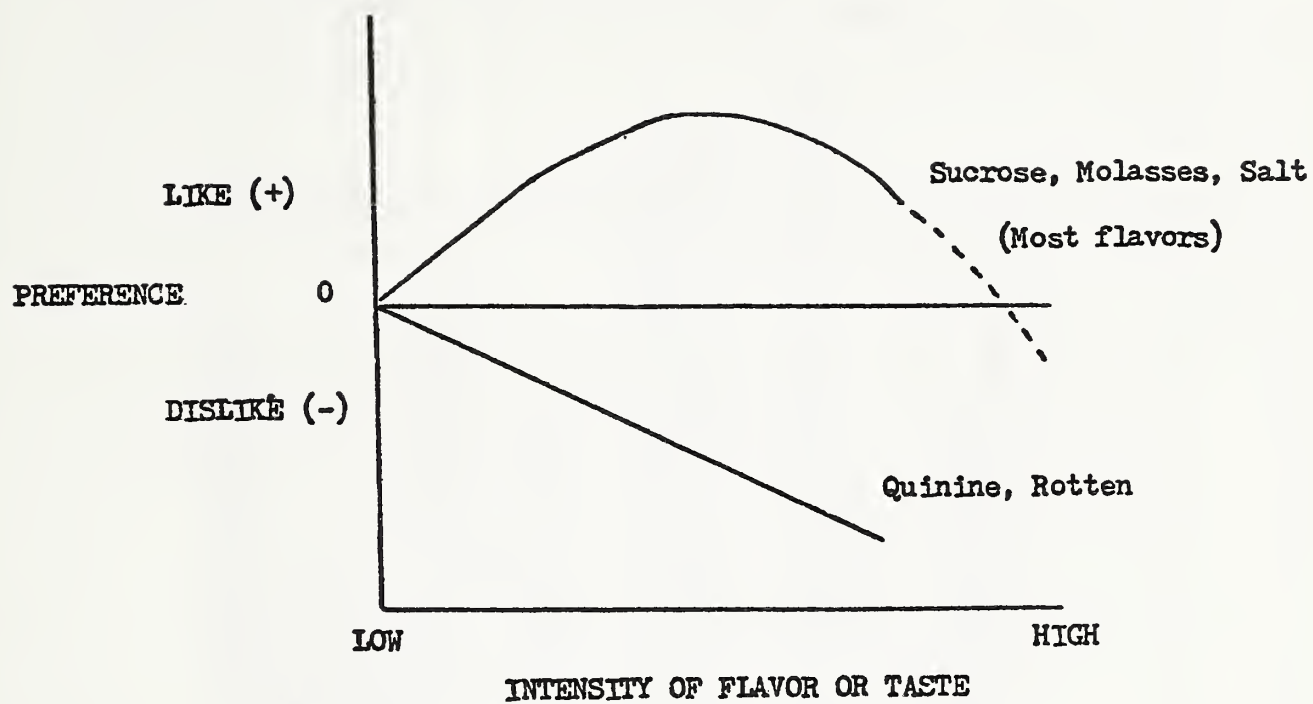


Figure 4.--Typical response curves for flavors. See text for discussion.

SENSORY PROFILES OF BROWN SUGARS

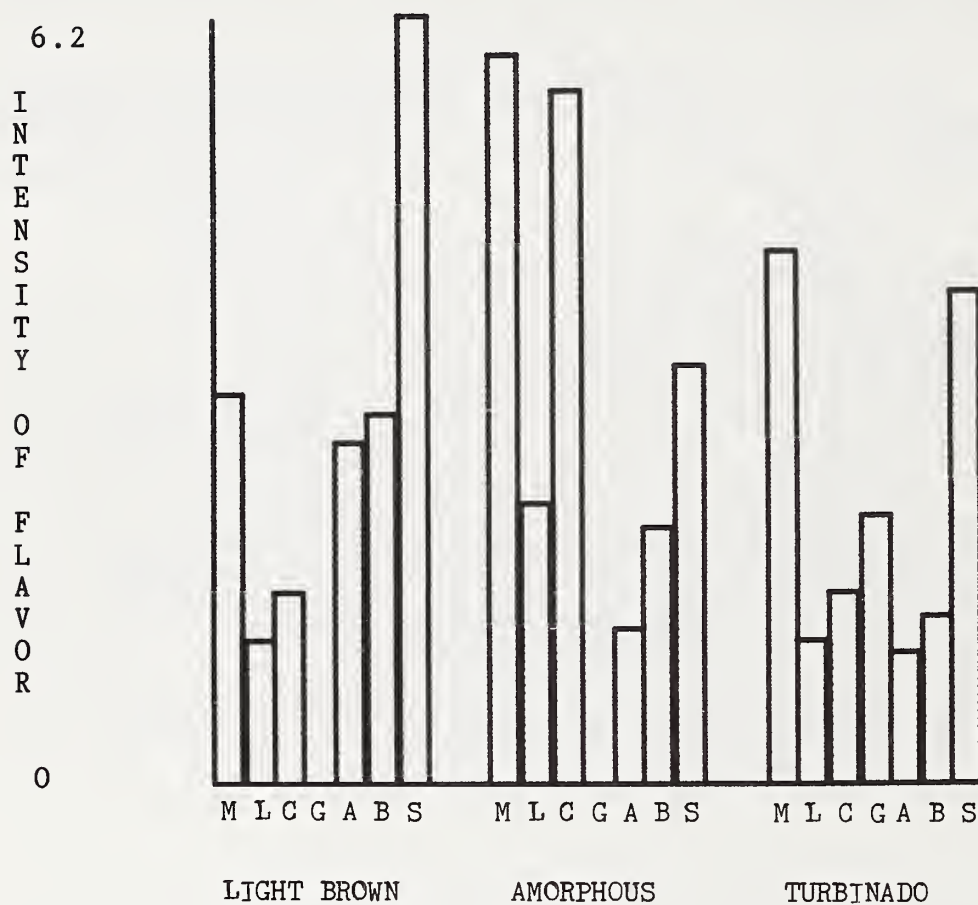


Figure 5.--Bar diagram profiles of several important flavor characteristics rated for 3 brown sugars. M = molasses, L = licorice, C = char, A = caramel, B = butterscotch, S = over-all flavor score.

Figure 5 shows a bar diagram profile of the four important flavor characteristics as they were rated for a light brown sugar (D-1), an amorphous sugar (N-3), and a turbinado sugar (L). Also included are caramel, butterscotch, and flavor score.

Flavor Scores by Category of Sugar. The flavor scores can be used to determine if type of sugar or type of process makes a difference in averaged scores. To do this, the flavor scores were ranked from highest score (rank=1) to lowest score (rank=27); sugars with the same score were given the same rank value. (For example, if sugars ranked 6 and 7 had the same flavor score, their rank value was each 6.5.) These ranks were averaged for category of sugar, and the results are shown in Table 4. The results show that turbinado sugars and amorphous sugars ranked quite low, while light coated sugars and Canadian light sugars ranked quite high. U.S. light sugars ranked about average, as did most of the other categories. Canadian dark sugars were on the low side. It is felt that the preference for Canadian light sugars may be due to their slightly stronger flavor. Possibly the Canadian dark sugars are too strongly flavored for American tastes. These results also point up one of the factors in flavor analysis--the results can often be influenced by regional preferences.

Since the number of samples for each category were so low, these results can only indicate trends. It is felt, however, that the low values for the turbinado sugars and the amorphous sugars are quite indicative of how much the processes for making these sugars differ from traditional soft sugars. Since turbinado sugars and amorphous sugars are very different from one another in their chemical composition (see Tables 5 and 6), the reasons for the low scores probably differ for each type.

Correlation of Flavor and Chemical Composition

Chemical Composition of Brown Sugars. The sugars were analyzed for acetic acid, titratable acidity, color, glucose, fructose, sucrose, phenol, iron, chloride, ash, and amino nitrogen. Table 5 lists the results for the turbinado sugars and Table 6 lists the results for the amorphous sugars. Differences are particularly evident between the two types of sugars with regard to all measurements except for amino nitrogen. The amorphous sugars had much higher values for these measurements than did the turbinado sugars. Both types of sugars had similar levels of amino nitrogen. The light and dark brown sugars had values higher than the turbinados also.

Volatile Profiles of Brown Sugars. Gas chromatography of the volatiles present in brown sugars revealed methanol, acetaldehyde, ethanol, methylpropanal, 2,3-pentanedione, methylbutanal, furfural and furfuryl alcohol, along with numerous smaller, unidentified components.

Table 4.--Average ranked flavor scores of brown sugars by categories

Category	Ranked score	No. of samples	Comment
Light Coated	7.1	5	very high score
Dark Boiled	11.1	6	--
Light Boiled	13.5	7	average score
Dark Coated	17.8	3	--
Turbinado	19.5	3	fairly low score
Amorphous	22.5	3	very low score

Simplified categories	Ranked score	No. of samples
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All Light Browns	10.8	12
All Dark Browns	13.3	9
Turbinado	19.5	3
Amorphous	22.5	3

Country of origin	Ranked score	No. of samples
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Canada Light	5.0	3
U.S. Light	11.1	6
Canada Dark	17.8	3
U.S. Dark	11.1	6
All Canadian	11.4	6
All U.S.	11.1	12

Table 5.--Composition of turbinado sugars

Sugar	Flavor Score	ppm Acetic Acid	Acidity	Color	% Glucose	% Fructose	% Ash
K	4.237	31	1.44	2482	.020	.073	0.19
L	4.00	72	0.72	1377	.023	.045	0.22
M	4.70	37	0.92	1504	.031	.034	0.22

Sugar	Phenol	ppm Iron	ppm Chloride	ppm Amino N	% Sucrose	% Invert
K	20.3	2.85	210	1280	99.5	.093
L	17.4	2.42	186	1280	100.4	.068
M	20.0	2.87	135	290	99.6	.066

Table 6.--Composition of amorphous sugars

Sugar	Flavor Score	ppm Acetic Acid	Acidity	Color	% Glucose	% Fructose	% Ash
N-4	4.50	1589	10.15	11613	1.04	0.67	1.41
N-5	3.43	0	2.83	6406	1.96	0.94	0.75
N-3	4.00	365	6.62	9791	0.90	0.66	1.32

Sugar	Phenol	ppm Iron	ppm Chloride	ppm Amino N	% Sucrose	% Invert
N-4	91.3	7.57	1569	150	97.1	1.71
N-5	69.2	7.98	951	1020	88.2	2.90
N-3	59.8	6.83	1899	200	92.5	1.56

Differences appeared in the volatile profiles of the different brown sugars. This is demonstrated in Figure 6, in which two light brown sugar volatile profiles are compared, one high in volatiles and one low. The sugar low in volatiles scored higher than did the high-volatiles sugar. Turbinado sugars were also low in volatiles, which can be seen in Figure 7, which compares a light brown sugar to a turbinado. However, the turbinado sugar, while low in most volatiles, was very high in acetaldehyde, methylpropanal, and methyl butanal, compounds that contribute green and fruity flavors. Both of the sugars in Figure 7 had fairly low flavor scores and also were rated for slight green and fruity flavor, so it is possible that these compounds may be useful in predicting quality.

A more instructive way to look at the volatile profiles is to compare individual peaks among several sugars, as shown in Figure 8, in which the acetaldehyde, diacetyl, and methylpropanal content of 6 sugars are compared in bar graphs.

Correlation of Flavor Scores with Volatile Constituents. Single linear regressions were run on flavor scores against the volatile constituents identified. The volatiles that correlated most highly with the flavor score of dark brown sugars (of 7 samples tested) were methylbutanal ($R=0.70$, $P=0.08$), furfural ($R=0.62$, $P=0.14$), and methylpropanal ($R=0.42$, $P=0.36$).

The volatiles that correlated most highly with the flavor score of light brown sugars (of 8 samples tested) were pentanedione ($R=-0.56$, $P=0.16$), diacetyl ($R=-0.44$, $P=0.28$), and acetaldehyde ($R=-0.43$, $P=0.30$).

Neither total volatiles, which included all identified and unidentified peaks, nor total acid volatiles correlated very well with flavor scores in this small set of samples.

Correlation of Flavor Score with Chemical Composition. Regression analysis was done of individual compositional variables on all the sugars as a group, all dark brown sugars as a group, and all light brown sugars as a group. The results for all the sugars and for the dark brown sugars are shown in Table 7. For all brown sugars, the flavor scores correlated best with amino nitrogen ($R=-0.57$), fructose ($R=0.45$), glucose ($R=0.41$), chloride ($R=0.38$), and ash ($R=0.36$). However, the correlations were low, and with a small number of samples, to be meaningful, correlations of 0.7 or better are needed, even if the lower correlations are statistically significant. Nevertheless, results such as these are important indicators of trends. Dark brown sugars flavor scores correlated best with phenol ($R=-0.52$) and amino nitrogen ($R=-0.43$).

When the correlations were done for the light brown sugars, a very different picture emerged. There were 12 traditionally processed light brown sugars (boiled or coated) and 2 amorphous sugars whose

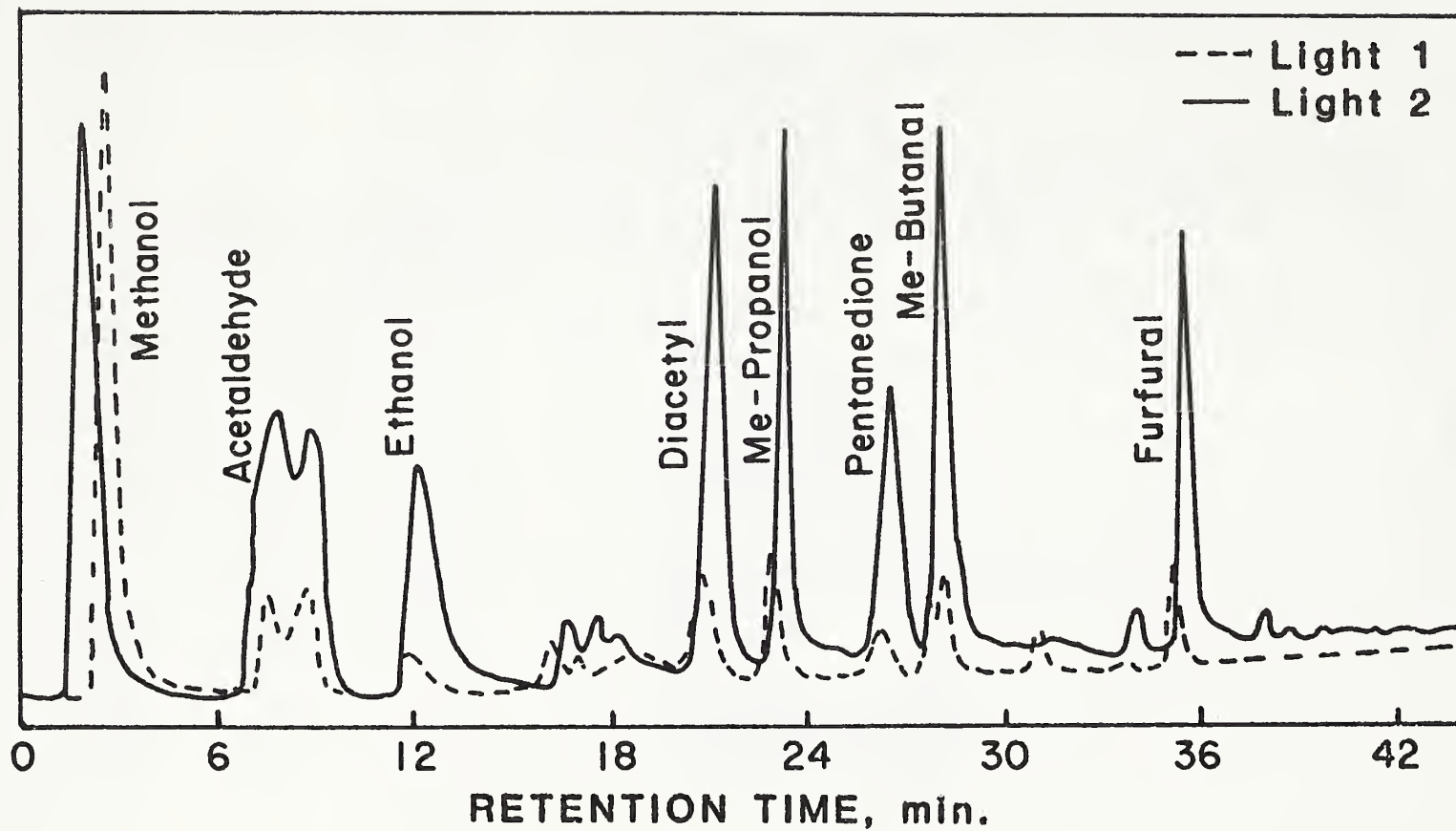


Figure 6.--Volatile profiles of two light brown sugars.

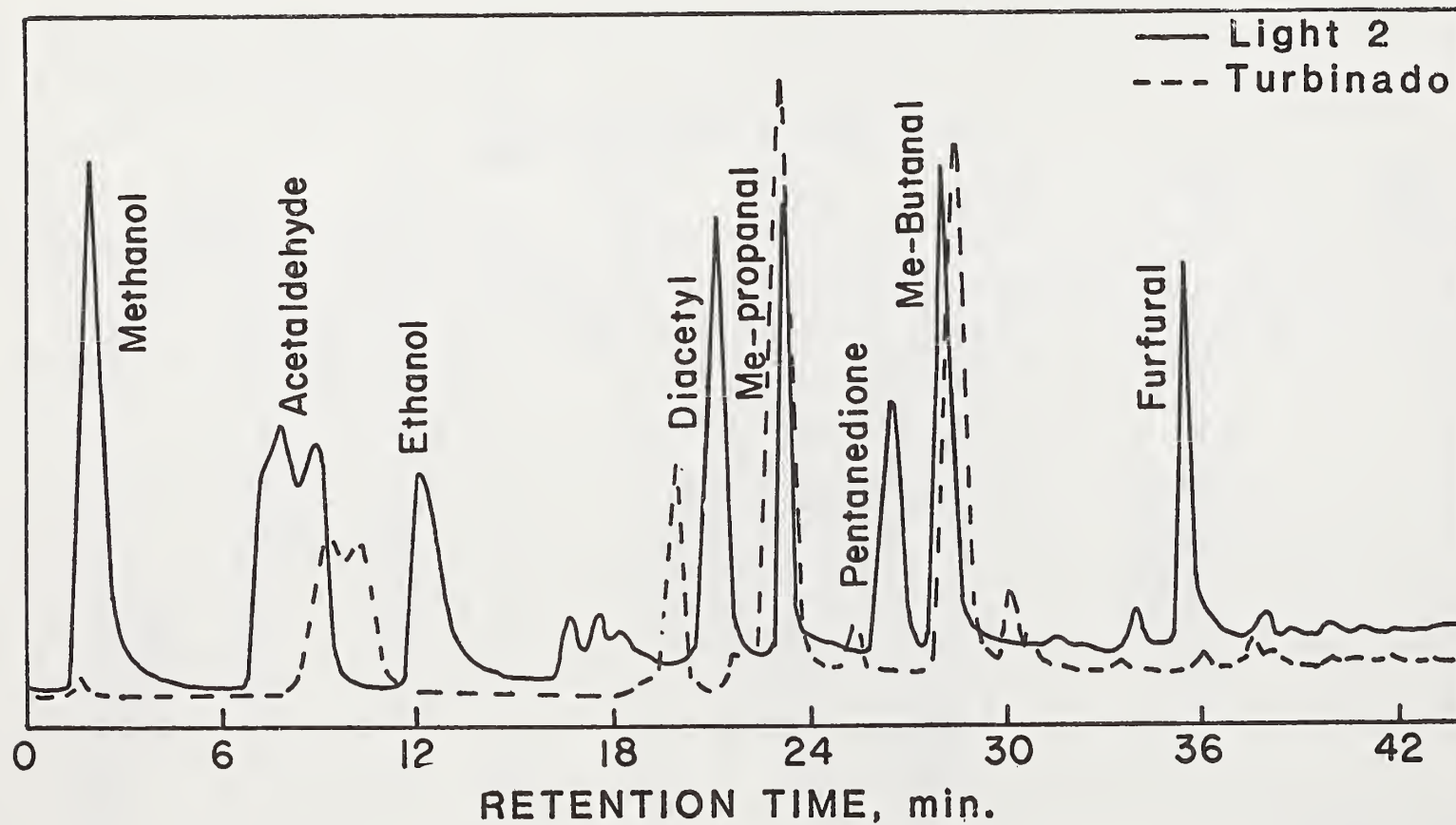


Figure 7.--Volatile profile of a turbinado sugar compared to the volatile profile of a light brown sugar.

COMPARISON OF VOLATILES IN SUGARS

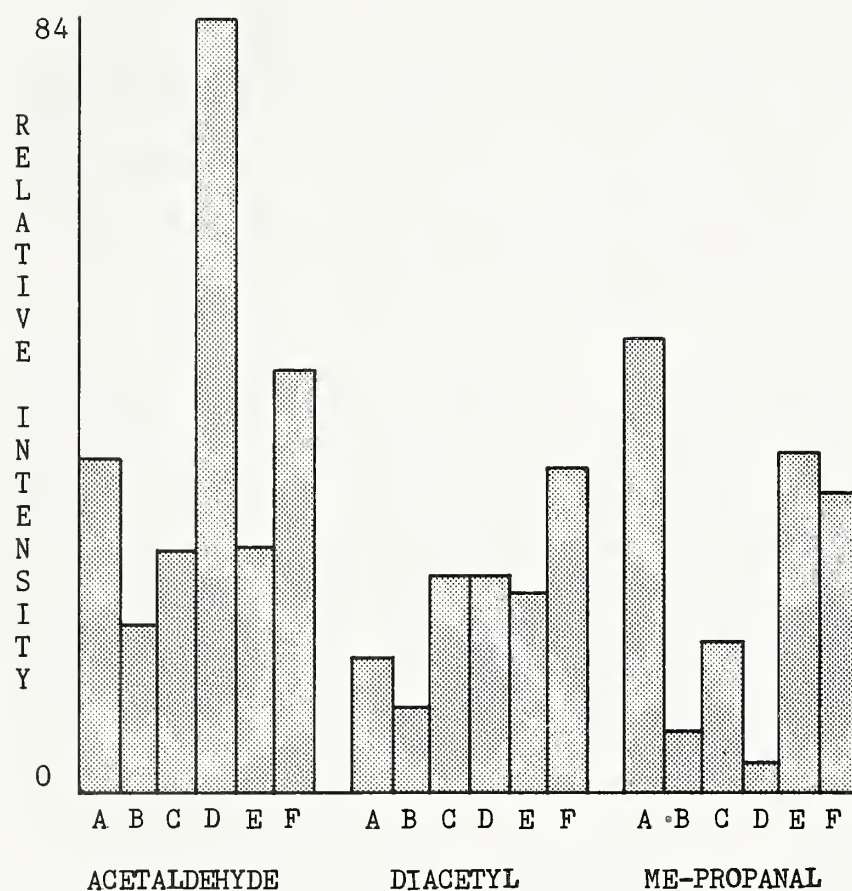


Figure 8.--Comparison of acetaldehyde, diacetyl, and methyl propanal content of six sugars. A = turbinado sugar (K), B = light brown sugar (D-1), C = light brown sugar (G), D = amorphorous sugar (N-5), E = amorphous sugar (N-4), F = light brown sugar (F-1).

colors would allow them to be placed in the light category. Single regressions were done on the 12 sugars and then repeated with the 2 amorphous sugars included. The results are shown in Table 8. Brown sugar flavor correlated much more closely with several parameters than did all the sugars together. Addition of the amorphous sugars to the calculations increased some of the correlations and did not change the trends in any significant way. This would indicate that (1) the amorphous sugars fell along the continuum for light brown sugars, although on the low end of the quality scale; and (2) these light brown sugars tended to be more consistent than the dark brown sugars. This is probably not surprising since some dark brown sugars have caramel color added to give them the required color, and in one case, invert was also added.

Multiple linear regression of the flavor scores of the light brown sugars was done since there were several significantly correlated variables. The 5 most significantly correlated variables were amino nitrogen, chloride, ash, color, and phenol. However, since chloride and ash were found to be highly correlated to one another ($R=0.95$), only one could be used in the model for predicting flavor score. We chose to use chloride since this showed the slightly higher correlation with flavor score. The results showed that the best predictors of flavor score were amino nitrogen and chloride, both with negative impact on flavor quality ($R^2=0.76$ for $N=14$ and 0.79 for $N=12$). Color, phenol, iron and invert were also included in the models, but the multiple regression showed that these variables were not significant in predicting flavor score.

Table 7.--Correlation of the flavor scores of brown sugars with chemical composition

<u>ALL BROWN SUGARS</u>			
Variable	R	No.	Significance level
Amino N	-0.57	24	1%
Fructose	+0.45	27	5%
Glucose	+0.41	27	5%
Chloride	-0.38	27	5%
Ash	-0.36	27	10%
<u>DARK BROWN SUGARS</u>			
Variable	R	No.	Significance level
Phenol	-0.52	10	20%
Amino N	-0.43	10	30%

Table 8.--Correlation of the flavor scores of light brown
sugars with chemical composition

Amorphous Sugars not Included (N = 12)		Amorphous Sugars Included (N = 14)	
<u>Significance Level*</u>	<u>R</u>	<u>Significance Level*</u>	<u>R</u>
<u>1%</u>		<u>1%</u>	
Amino Nitrogen	-0.74	Amino Nitrogen	-0.84
Color	-0.69	Chloride	-0.82
Phenol	-0.67	Conductivity Ash	-0.75
<u>5%</u>		<u>5%</u>	
Chloride	-0.64	Phenol	-0.69
Conductivity Ash	-0.63	Color	-0.60
<u>10-20%</u>		<u>10-20%</u>	
Fructose	+0.49	Fructose	+0.48
Invert	+0.41	Invert	+0.46
Iron	-0.41	Iron	+0.46
		Glucose	+0.43
<u>Not Significant</u>		<u>Not Significant</u>	
Acetic Acid		Acetic Acid	
Glucose		Acidity	
Acidity		Volatiles	
Volatiles			

* Correlations should be 0.7 or better with small samples to be meaningful even if statistically significant.

CONCLUSIONS

In summary, this study has shown that it is possible to conduct sensory evaluation of brown sugars and to obtain a great deal of information about the sensory qualities that correlate with chemical composition.

The evaluation of brown sugars is complicated by the sugar matrix. Sugar masks other flavors and modifies them, and some subthreshold effects were apparent for several added flavors. Compounds with negative taste attributes, such as the bitter compounds caffeine and naringen, elicited positive descriptors when they were present in concentrations below the recognition level. At higher concentrations, these compounds elicited the appropriate negative descriptor. This data suggests that constituents such as chloride, amino nitrogen, and phenolics are necessary to round out the flavor of brown sugar, but must be present at subthreshold levels. This would also explain why, in a previous study, brown sugar extracts obtained with XAD-2 resin, that had extremely bitter flavor, could produce a brown sugar flavor when mixed with white sugar (Godshall, 1983).

In this study the sugars were rated for 13 flavor attributes. Analysis of the results showed that 4 of these attributes were significant, and 2 or 3 other attributes provided useful information. The significant attributes were char, licorice, molasses and green. Contributing information but not statistically significant were caramel, butterscotch and fruity flavors. Char and licorice flavors were the most significant predictors of the flavor score. Green flavor was an important attribute in determining if the sugar had raw sugar taste, such as the turbinado sugars. In future sensory evaluation of brown sugars, a much smaller number of attributes could be rated, thus simplifying the task of the panelist.

Since the flavor scores showed significant differences among sugars, it was possible to also determine if processing affected flavor. Turbinado and amorphous sugars were found to rate lower scores, on the average, than the other sugars.

Chemical analysis of the sugars showed that turbinado sugars differed markedly from other brown sugars in having much lower concentrations of all constituents tested with the exception of amino nitrogen, which was higher. The amorphous sugars tended to have high amino nitrogen also, but their chemical composition was more similar to the other brown sugars.

Several chemical variables correlated significantly to flavor score, particularly amino nitrogen. Light brown sugars showed the stronger correlations, indicating more consistency in their manufacture. Multiple linear regression of the significantly

correlated measurements with the flavor score showed that the best predictors of light brown sugar flavor score were amino nitrogen and chloride. Other variables such as phenol and iron did not significantly increase the correlation. In summary, low amino nitrogen and chloride content are desirable for good brown sugar flavor.

ACKNOWLEDGEMENTS

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DISCUSSION

Leon Anhaizer, Imperial Sugar: You've come a long way in the data, and it's really a fine bit of work you have done. I'd like to ask a question, because when you present all this data, it reminds me of an old song, "Save the last dance for me." Did you try to organize the presentation of flavors to the taste buds so that you could see what their influence is in the final sensory evaluation because we all know about after taste and the last taste, like the last dance. It's that last impression. Did any of your work consider the effect of the last flavor on the sensory evaluation?

Godshall: A lot of sensory evaluation does consider the order in which flavors are perceived and the after taste. We did not ask for that information in the presentation of the samples nor in the training. In the training we did ask if an after taste was noted but got little response to this question and so dropped it.

Steven J. Clarke, Audubon Sugar Institute: I've noticed that the flavor of fresh raw sugar is quite different from raw sugar that has been stored a long time. Were the samples of turbinado all the same age when your panel tasted them, and did you have a look at the effect of storage on the shelf life of the flavor?

Godshall: We've done a little work on the effect of storage on flavor but don't have data to present. It's of a qualitative nature. The turbinados were all obtained at the same time. The commercial turbinado, Sugar-in-the-Raw, was bought at the store and we don't know how old it was, but the other two were fresh. The fresh ones had a strong "green" flavor, as a matter of fact, with Sugar-in-the-Raw having less of this flavor, but still quite perceptible. The gas chromatography profiles of the volatiles in the turbinados showed the presence of the alcohol, cis-3-hexenol, which is responsible for green and grassy flavors, and which comes from the leaves of the sugarcane plant.

Stanley E. George, B.C. Sugar: Your last slide showed a lot of flavors, char, licorice, etc. Are these bad or good, and which are which?

Godshall: Char and licorice correlated negatively to the flavor score, so we would have to say that these tend to be undesirable. You would not want a very strong indication of either of these flavors in your product. Caramel flavor correlates positively and is thus desirable. Molasses is desirable if the flavor is not too strong, which would then have a negative flavor impact.

George: My next question is, in view of all this, what do you think of the old statement that you can only make good soft sugar from char treated liquor?

Godshall: We would like to get samples of soft sugars that were not char treated. All of ours were except for the amorphous and turbinado sugars, of which both types fared poorly in the scoring.

George: Another more specific question: If a refinery, and B.C. Sugar happens to be one, is changing its process, in our case, from straight char or char-carbon (Canesorb System) filtration, to adding phosphatation, could these sensory techniques be used to determine if it was going to do something, either good or bad to our soft sugars? In our case, soft sugar is 14% of our production so it is an important element in our output.

Godshall: Quantitative descriptive flavor analysis such as I have described in this study would not be applicable to your situation. This technique is expensive and complex and gives you a lot of data and information which is helpful in understanding the overall contributions to flavor. In the case where you are changing a process and you mainly want to know if the new process is the same as or different from the old process, I would recommend the triangle test or a preference test, either one of those. The triangle test is particularly recommended. It can be done in-house and doesn't require the degree of training that QDA does. It will tell you if there is a difference, how much difference exists, and what form the difference takes.

George: You say you don't have to use trained people. Would you not have to have them desensitized? We had some tests done some years ago when we were looking at making some modifications to our Golden Syrup production methods, and we went to the University of British Columbia. They used 5 or 10 people, but they weren't used to tasting 81 Brix Golden Syrup and it took them a while to overcome the sweetness and to concentrate on the other flavors.

Godshall: Absolutely. There is a process of familiarization with the product which takes some time. You will find that people who work with the product, such as refinery people, are already quite

familiar with the product and are very highly motivated to perform the sensory task, more so than someone else such as university people, who first have to become familiar with the product. This is like a type of training but does not involve formal training sessions and is more of a familiarization process. The triangle test can be done in-house. You could use 20 to 30 people and so the triangle test several times and find out if there is a difference and how different it is.

RECENT OBSERVATIONS ON STARCH AND SUGARCANE PRODUCTS

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INTRODUCTION

The occurrence of starch in sugarcane has been known for over fifty years (Haddon, 1926). Starch is present in the form of granules of characteristic size and shape which are carried away from the plant tissue and into the juice during the milling process. In subsequent steps in sugar processing, starch granules are gelatinized and some of the solubilized starch becomes occluded in the raw sugar crystals (Alexander and Matic, 1974). It is of interest to note that as long ago as 1927 a process was patented for using thermally stable enzymes to hydrolyze starch in sucrose manufacture (Haddon, 1927).

Vignes (1974) isolated starch granules from sugarcane and examined them by both light and electron microscopy. This study of starch granules in raw sugar, a continuation of our interest in the role of various polysaccharides in sugar processing, describes microscopic examination of the granules.

EXPERIMENTAL

The five raw sugars used were commercial materials originating from outside the USA.

Raw sugar solution at 50° Brix was centrifuged at 14,000 rpm (44,000 g) for 20 minutes in a Sorvall superspeed centrifuge. The solution was decanted, and the residue was washed twice with water with recovery of solids each time by centrifuging at 14,000 rpm for 10 minutes. The residue was freeze-dried prior to microscopic examination.

Polarized light microscopy was performed using an Olympus Vanox polarizing microscope (Olympus Optical Co., Tokyo, Japan) with a camera attachment. Gelatinization temperature of starch granules in water or sucrose solutions was determined on a hot stage attachment to the polarizing microscope by the procedure of Schoch and Maywald (1956).

Slide-mounted material was stained with 0.1 M iodine in 10% potassium iodide in order to detect starch granules or with Nile Blue A to detect hexane-soluble lipids (Hargin, et al., 1980). When Nile Blue A was used the stained material was examined for fluorescence using a fluorescence vertical illuminator attachment to the microscope.

RESULTS AND DISCUSSION

Starch granules occur in plastids in all parts of most plants (Badenhuizen, 1965). Some granules are transitory whereas others function as long-term storage reserves. Unlike transitory granules, reserve granules generally possess a shape and a size range characteristic of the organ of the plant species from which they were isolated. The main component of the starch in the granule is amylopectin, an α -D-glucose polymer containing mainly 1,4-linkages with about 5% 1,6-linkages; amylopectin has a molecular weight of about 500 million, and comprises about 100,000 unit chains arranged in the form of a highly-branched tree. Accompanying amylopectin is another α -D-glucose polymer, amylose, containing mainly 1,4-linkages with a few (0.1-0.2%) 1,6-linkages. Amylose, which is an essentially linear polymer, has a molecular weight of about one million. These two glucose polymers are combined in the starch granule as a partially crystalline glassy material.

Sugarcane starch granules are almost spherical and smooth with an average diameter of 5 μ m (Vignes, 1974; and Reichert, 1913). They contain 19-21% amylose, a level found in a number of other plant starches; e.g., wheat starch contains 21-24% amylose. The percent amylose increases with increasing age of the tissue from which the starch is derived.

In the five raw sugar samples which we have examined, two sizes of starch granule were observed under the polarizing microscope. Larger granules of average size 5 μ m were accompanied by smaller granules of average size 1 μ m. The smaller granules were attached to plant vascular material (Figure 1) whereas the larger granules were completely free

of any other visible material. Both types of granule showed the typical Maltese cross observed with starches organized in granules (Schoch and Maywald, 1956). This birefringent pattern is derived from the radial orientation of crystalline regions containing amylopectin (Badenhuizen, 1965). Only hesperidin crystals in citrus and some types of cellulose in wood pits are known to give a birefringent pattern similar to the Maltese cross of starches.

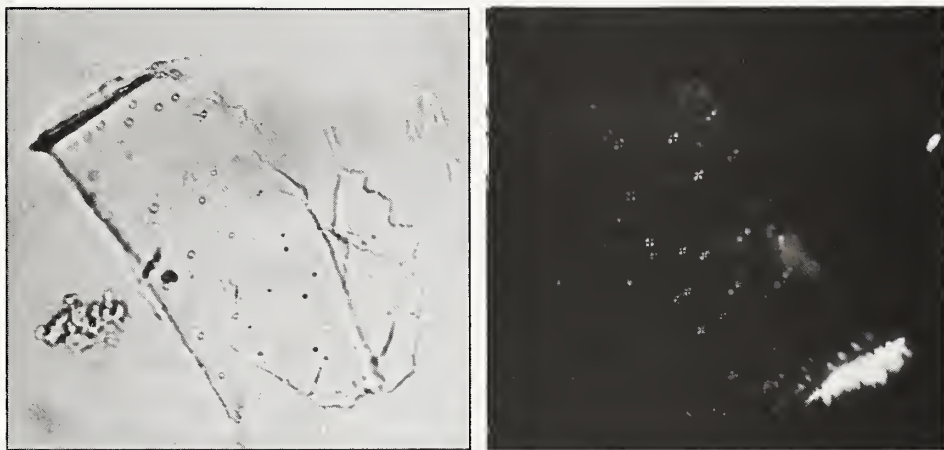


Figure 1--Photomicrographs of $1\mu\text{m}$ starch granules attached to plant vascular material under unpolarized (left) and polarized light (right).

Generally, starch granules show a size distribution having a Gaussian distribution, but those of some cereals are bimodal (Bathgate and Palmer, 1972; Sandstedt, 1946; Sandstedt and Schroeder, 1960). Wheat starch consists of lens-shaped granules of mean diameter $25\mu\text{m}$ and spherical granules of mean diameter $5\mu\text{m}$. The two types of granules are formed in the same cells in the wheat endosperm (Sandstedt, 1946; Sandstedt and Schroeder, 1960). The large granules in wheat starch comprise 18% by number and 96% by weight of the total starch (Bathgate and Palmer, 1972), contain 0.1% protein, and have a mid-point gelatinization temperature (T_g) of 61°C . In contrast, the small granules contain 1.6% protein, and have a mid-point T_g of 72°C . Both types of granule have the same amylose content of 24-25% (Bathgate and Palmer, 1972). Barley starch granules have similar physical and chemical properties to those of wheat starch, except that large barley granules have an amylose content of 25% and the small granules 41% amylose (Bathgate and Palmer, 1972). It has been suggested that the two types of starch granules are

synthesized and deposited by different mechanisms which include the formation of one or more than one granule in each plastid (Buttrose, 1960; Meredith, et al., 1981). The small granules are formed at a later stage than the large granules.

The large granules from the raw sugars had Tg values in water and sucrose solutions in line with those of other starches containing about 20% amylose (Table 1). The mid-point Tg values ranged from 58-106° for 0-70° Brix sucrose solutions. The loss of birefringence and swelling of the starch granule occurs over a small temperature range (2°C) for each individual granule, but with the large population of granules observed in the field of view of the polarizing microscope the individual variation in gelatinization results in a temperature range of 8-10°C over which birefringence is lost. At this point the granule has become a swollen gel particle and dissolution has not occurred.

Table 1.--Temperature Range for Loss of Birefringence by Cane Starch in Sucrose Solution

Temperature, °C, for % loss of birefringence			
<u>Brix</u>	<u>2%</u>	<u>50%</u>	<u>98%</u>
0	56	58	59
10	58	61	65
20	60	64	70
30	65	70	74
40	73	76	79
50	81	85	88
60	93	95	97
70	103	106	--

In contrast to the large granules of sugarcane starch, the small granules did not gelatinize in water or 15° Brix sucrose solution when heated up to the boiling point of the solution, and did not stain with iodine.

The phase transitions which occur during the heating of starch granules in aqueous solutions are best followed by differential scanning calorimetry (DSC) (Wong and Lelievre, 1982). The transitions in starch gelatinization include penetration of water molecules into starch crystallites,

glass transition of amorphous material, melting of starch crystallites, and swelling of crystalline and amorphous material (French, 1984). We have yet to perform DSC measurements on either type of raw sugar starch granules because, although the quantity of starch granules needed for DSC is small, removal of extraneous particulate material from the granules is not easy.

A number of possible explanations of the presence of two sizes of starch granules in raw sugar can be offered. Because we have found the two types of granules in raw sugars of different origins we have rejected the idea that the granules are from a plant other than sugarcane. It is unlikely that one type of granule is from sugarcane leaf and the other from stem, but the possibility of size differences in granules from nodes and internodes needs to be examined. The different granules could arise from different methods of synthesis (cf. Meredith et al., 1981), e.g., involving different enzymes or substrates, or from differences in physiological age of the plant material leading to differences in the ratios of amylose: amylopectin (Cashen and Friloux, 1966). In the corn sweetener industry, starch granules have been found in both enzyme- and acid-hydrolyzates of corn starch (Hebeda and Leach, 1974). The granules were shown to be degraded amylose in the acid hydrolyzates, and complexes of amylose with free fatty acids in the enzyme hydrolyzates (Hebeda and Leach, 1974), and they were derived from retrogradation of amylose. In the case of raw sugar it is unlikely that retrogradation of amylose is responsible for the occurrence of small granules because the concentration of dissolved starch in sugar liquor is so low. Another possible explanation is that the small granules are associated with protein or lipid. Small granules have been treated by a Niles Blue A staining technique (Hargin et al., 1980) in order to detect hexane-soluble lipid association with the granules. There was some sign of yellow fluorescence which would indicate non-polar lipid, but at this time the result is not conclusive.

The effect of the two types of starch granules in sugar processing is clear cut. The larger granules will gelatinize under process conditions and will contribute to the problems described by Alexander and Matic (1974). The smaller granules do not change under process conditions and become part of the filtration problem.

ACKNOWLEDGMENT

The authors wish to thank Jarrell H. Carra for photomicrography.

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DISCUSSION

Stanley E. George, B.C. Sugars: Are there differences in the pattern of appearance of this starch based on certain areas or locations where the sugar comes from?

Parrish: We found these two types of granules in all five raw sugars we looked at. At this point, we are not making any comparisons as to which locations are worse than others because we don't have numbers on the amounts of these granules. You can appreciate that where one granule is five times the size of another one, on a weight basis that is a factor of 5 cubed, 125, so while there are many, many small granules, you would need 125 small granules to equal the weight of one large granule. We haven't got those numbers at this point. We have a total starch analysis, but then the small granules are not picked up in the starch analysis.

A GLUCAN FROM SUGARCANE

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INTRODUCTION

Sugarcane contains several types of polysaccharides; among them are starch, which is a mixture of amylose and amylopectin, and another type which we have designated indigenous sugarcane polysaccharide (ISP). These polysaccharides affect sucrose yield, the efficiency of crystallization, press filtration, clarification and other process steps.

The composition of ISP may vary with cane variety, the method of purification and the age of the cane from which it is extracted. Upon acid hydrolysis ISP yields arabinose, xylose, rhamnose, mannose, galactose, and glucose (Roberts et al. 1976). Glucuronic acid has also been found to be a component of ISP (Roberts and Godshall 1978a). No galacturonic acid has been found. It is now thought that the galacturonic acid reported to be in sugarcane in early literature (reported present as pectin) was actually glucuronic acid misidentified as galacturonic. There is galacturonic acid (as pectin) in beets and beet sugar; but none, apparently, in cane. ISP is the most abundant organic non-sugar in cane juice other than organic acids and amino acids (Clarke et al. 1978). We have shown that ISP is one of the causative factors in the formation of acid beverage floc (Roberts and Godshall 1978b). The effects of ISP on sugar processing are recognized qualitatively, but are not known quantitatively.

Several investigators have reported the appearance of two peaks when ISP was chromatographed on gel filtration columns (Vane 1982, Blake et al. 1983), indicating the presence of two fractions of different molecular weights; however, the compositions of these peaks were not reported.

Bruijn (1966, 1973) reported the isolation from stale cane of a glucan which he called sarkaran. This glucan reportedly was

composed of groups of 3 and 4 glucose units with α -(1-4) linkages (maltotriose and maltotetraose) and these groups were linked together by α -(1-6) linkages. Blake and Littlemore (1984) here isolated a similar polysaccharide from standover cane (cane harvested one crop year late) which they identified as sarkaran, composed of almost equal quantities of maltotriose and maltotetraose combined in α -(1-6) linkages. Cuban workers (Cremata and Orozco 1980, Cremata et al. 1983) have isolated a polysaccharide from molasses, and proposed a 1-3 glucan main chain with α -(1-6) side chains and some arabinose side chains. This polysaccharide, like that identified by Blake and Littlemore (1984), is thought to cause high viscosities in syrups and process streams. Cremata and coworkers (1983) observed that their polysaccharide caused crystal elongation along the C-axis, as does dextran.

In this paper we report the separation of a glucan from ISP from fresh cane. Evidence for the structure ascribed to the glucan is also presented.

EXPERIMENTAL - METHODS AND MATERIALS

Separation of ISP. The ISP was separated from the juice of 450 lbs fresh cane, as described by Roberts, Jackson and Vance (1964). Cane was in early maturity (11 months), machine cut (soldier harvester) and hand cleaned.

The ISP was prepared from cane which was cut and ground in an experimental mill located at the edge of the cane field, so that the maximum time that expired between cutting and grinding the cane was 2 hours, at temperatures below 20° C. The cane was crushed and macerated during a period of about 30 min; mixed juice was poured into a tank and immediately filter aid and acetic acid were added along with sufficient absolute ethanol to produce an alcohol content of 75%. The precipitate was allowed to settle over night. The supernatant liquid was syphoned off. The precipitate was filtered off, dried and the ISP was extracted as indicated. By this procedure, no time was allowed for the action of microorganisms and the formation of dextran. Starch was eliminated in the extraction of the ISP from the precipitate. The yield of ISP was 29.5 g from 450 lbs. cane.

Purification of ISP. A 250 g portion of the filter aid, containing the ISP, was suspended in 800 ml of water and homogenized for about 30 seconds in a high speed blender to break up lumps. The suspension was then heated to boiling and filtered with suction on Whatman No. 4 paper. The filter cake was suspended in 500 ml of water, heated to boiling and filtered as before. The filter cake was again suspended in 500 ml of water, heated to boiling and filtered. The filter cake was discarded. The combined filtrates were concentrated under vacuum to 200 ml. The solution was

deproteinated by the addition of 20 ml of 5% ZnSO_4 solution and 17.6 ml of 0.4N Ba(OH)_2 . The solution was filtered on a filter aid mat, or centrifuged, and then dialyzed in a 12,000 molecular weight cut-off bag against flowing toluene-saturated deionized water for 100 hrs. The solution remaining in the bag was evaporated under vacuum to 100 ml. The solution was decolorized by stirring for 15-20 minutes with 5 g of DEAE cellulose. The DEAE cellulose was removed by filtration and the filtrate was freeze dried. The yield of purified ISP was 5 to 7 g, from 250 g of the filter aid mixture.

Separation of the glucan from ISP. Five grams (5g) of ISP was dissolved in 500 ml of deionized water and placed in the reservoir of a hollow fiber dialyzer (Amicon Corp., Lexington, Mass.) with a 50,000 MW cut-off cartridge. The apparatus was connected to an external vessel containing 300 ml of water and the dialysis was carried out at a rate of 200 ml per minute. The water in the external vessel was changed six times during 30 hr of dialysis. The external solutions were combined and evaporated under vacuum to 50 ml and freeze dried. The yield of material passing through the 50,000 MW cut-off fibers was 2.0 g (40% of the ISP).

Specific Rotation. Specific rotation was determined on a Perkin Elmer 241 MC automatic polarimeter.

Acid hydrolysis of the glucan. An amount of 0.5 g was hydrolyzed with 2N sulfuric acid as described by Roberts and Godshall, (1) and subjected to GC analysis.

GC Analysis of hydrolyzed glucan. GC analysis of the trimethylsilyl ethers of the acid hydrolyzed glucan was carried out on a Hewlett-Packard chromatograph model 5880 with a fused silica capillary column 12 meters in length coated with OV-101. The operating temperature was 175°C for four minutes, then programmed at 4°C/min .

Methylation of the glucan. An amount of 1.0 g of the glucan was methylated by the method of Hakomori (1964).

Hydrolysis of the methylated glucan. The methylated glucan was hydrolyzed by boiling under reflux in 2N sulfuric acid for 16 hr. The sulfuric acid was removed with barium hydroxide. The solution of the hydrolyzate was concentrated and freeze dried.

Reduction of the hydrolyzate. An amount of 0.5 g of the hydrolyzed methylated glucan was reduced to the methylated alditol with sodium borohydride as described by Roberts and Rowland (1970).

GC Analysis of the methylated alditol. GC analysis of the methylated alditol obtained from the methylated glucan was carried out on a Tracor chromatograph model No. 565. The column was a 30 meter capillary column coated with OV351. The operating

temperature was 175° C programmed at 4° C/min to 240° C. Additional methylation analysis was carried out on the Hewlett-Packard GC system described above.

Amylolysis of the glucan. An amount of 0.2 g of the glucan was dissolved in 5 ml of buffer at pH 5.0 with an excess of α -amylase (Mycolase, GB Fermentation Industries; fungal α -amylase from *Aspergillus oryzae*), and the solution incubated overnight at 40° C. The hydrolyzate was subjected to thin layer chromatographic analysis, as below.

Pullulanolysis of the glucan. An amount of 0.2 g of the glucan was dissolved in 5 ml of buffer at pH 5.0 and an excess of pullulanase (Sigma Chemicals; from *Enterobacter aerogenes*) was added and the solution was incubated overnight at 40° C. The hydrolyzate was subjected to TLC analysis and HPLC analysis.

Thin layer chromatography of the pullulanolyzate. The pullulanase hydrolyzate was analyzed by thin layer chromatography using glass plates precoated with silica gel without fluorescent indicator. The developing solvent was n-butanol-ethanol-water-acetic acid in the ratio of 50: 30: 15: 5. The hydrolyzate was applied to the plate which was developed for 2 hr. The plate was dried, sprayed with 2N sulfuric acid and heated in an oven at 105° C for 10 minutes (Roberts and Godshall 1984).

Amylolysis of the fraction not hydrolyzed by pullulanase. The unhydrolyzed material and silica left at the origin of several developed and unsprayed plates was scraped off and treated at pH 5.0 with α -amylase. The hydrolyzate was analyzed by TLC.

HPLC Analysis: High pressure liquid chromatography was conducted on a Waters Associates (Milford, Mass.) Model 6000 A solvent delivery system, with a R-401 Differential Refractometer detector, WISP Automatic injection system, and a Model 730 Data Module for recording and integration. The column, (Waters Associates), was Dextropak in a radial compression (RCSS) system, with water at ambient temperature as solvent.

Viscosity measurement. Viscosities of aqueous and 60 Brix syrup (both raw and refined sugars) solutions of the glucan were measured at 20 ppm glucan on an oscillation viscosimeter, Model 7.006 (Nametre Co., Edison, N.J.). The instrument was zeroed in air and calibrated with water.

Polarized light microscopy. The glucan was examined under magnification in polarized light on an Olympus Vanox polarizing microscope (Olympus Optical Co., Tokyo, Japan).

Periodate oxidation. Periodate oxidation was performed on the polysaccharide; periodate consumption was measured (Aspinall and

Ferrier 1957, Hay et al. 1965) and formic acid and formaldehyde produced (Parrish 1958) were analyzed.

Beverage floc potential. The glucan was tested for acid beverage floc potential by the Coca-Cola and the Australian tests (Clarke et al. 1980).

RESULTS AND DISCUSSION

The glucan, separated from the ISP by dialysis in an Amicon hollow fiber dialyzer with a 50,000 MW cutoff cartridge, was prepared in yield of 40% of the ISP. It showed a specific rotation $[\alpha]_D^{25} +120^\circ$ which indicates that its glucose units are α -linked. Upon acid hydrolysis, the glucan yielded about 98% glucose with traces of arabinose, galactose, and mannose. The glucan produced a red-purple color with iodine, similar to that of amylopectin.

Under polarized light, and magnification up to 500x, the glucan exhibited very slight birefringence, but no Maltese cross pattern was observed as is found in starch granules.

The glucan was permethylated, the methylated product was reduced to the alditol, and the alditol was analyzed by GC. Comparison with the GC analysis of known 2,3,6-O; 2,4,6-O; and 2,3,4-O-trimethyl glucose derivatives and the tetramethyl glucose derivative showed that the glucan was predominantly α -(1-4) linked with some α -(1-6) linkages, and a relatively high percentage (11-13%) of terminal groups.

Periodate oxidation showed about 1 mole periodate consumption per molar proportion anhydroglucose unit, with production of a slight amount of formic acid or formaldehyde; this is consistent with the structure from methylation data.

Viscosity measurements showed that the glucan produces only a very slight increase in viscosity upon addition to water, raw sugar syrup or refined sugar liquor. Levels are shown in Table 1. The increase was greater upon addition to raw sugar syrup, as was expected because of the interaction with ash components. Sugar solutions were prepared by weight; hence, the raw shows a lower viscosity than the refined in Table 1.

Beverage floc tests were negative: the glucan exhibited no floccing potential.

Treatment of a solution of the glucan with α -amylase at pH 5.0 yielded only glucose as shown by TLC analysis. The α -amylase apparently contained α -glucosidase, converting maltose and maltotriose to glucose.

Table 1.—Viscosity (cps) of solutions of the glucan at 25° C

	Water	Viscosity, CPS	
		60 Bx sugar (refined)	60 Bx sugar (raw)
Without glucan	0.812	45.0	38.1
20 ppm glucan added	0.827	44.7	39.8

Treatment of a solution of the glucan with either pullulanase or isoamylase yielded glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, and maltooctaose as indicated by TLC analysis and HPLC analysis, with maltotriose, maltotetraose, and maltopentaose predominating, as shown in Figure 1. Some carbohydrate material remained at the origin. This was scraped from the plate and when treated with α -amylase yielded only glucose.

Since pullulanase and isoamylase are debranching enzymes and cleave only α -(1-6) linkages, and since the material remaining at the origin yields only glucose when treated with α -amylase, it is concluded that the glucan has an α -(1-4) linked straight chain backbone, with side chains of α -(1-4) linked glucoses ranging in length from glucose to maltooctaose, linked to the backbone by α -(1-6) linkages, as shown in Figure 2. Possible structures are shown in Figure 3.

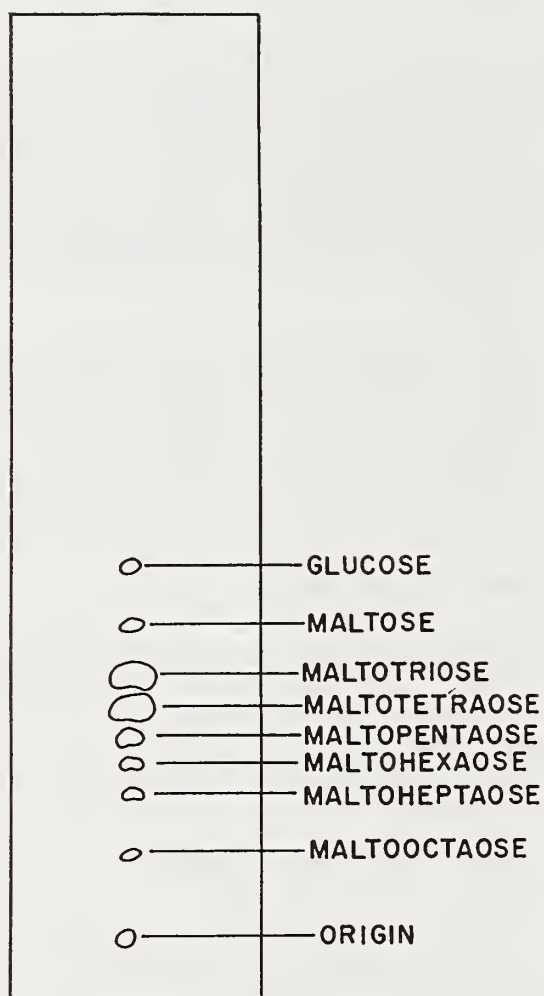
The high percentage (about 12%) of terminal groups indicates a high degree of branching, and establishes that this glucan is different from amylopectin in degree of branching (5%). The highly branched structure accounts for the very soluble nature in water of the glucan, and also for the lack of effect on viscosity, either in water or in raw or refined sugar solutions.

SUMMARY AND CONCLUSIONS

A glucan having a mw of 15,000–50,000 was isolated from cane juice. The high positive rotation of the glucan indicated the linkages were in α -configuration. GC analysis of methylated alditols obtained from the methylated glucan along with known methylated alditols showed that the glucan was predominantly α -(1-4) linked with some α -(1-6) linkages. Periodate oxidation data was in agreement with the methylation data. The large percentage of terminal groups shown by methylation explained the high degree of solubility of the glucan, as well as its low viscosity.

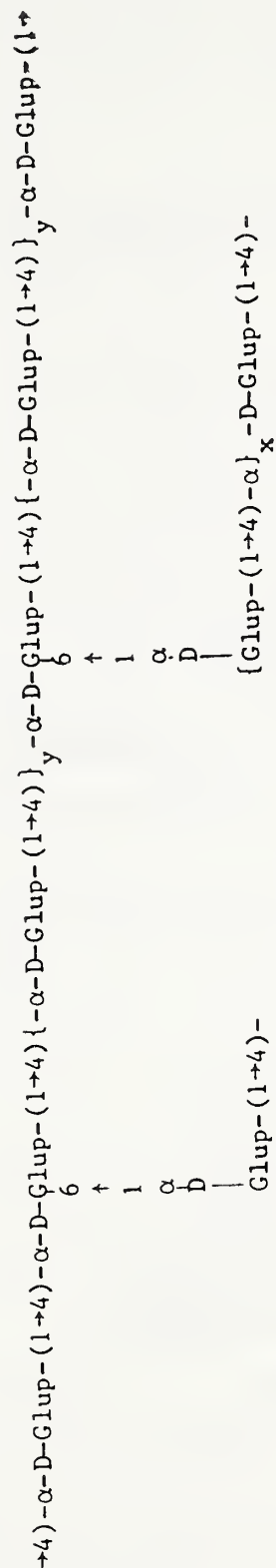


a



b

Figure 1.--(a) Thin layer chromatogram of pullulanase hydrolyzate of sugarcane glucan and maltotriose (MT); (b) diagram of thin layer chromatogram of the pullulanase hydrolyzate of the glucan.



$x = 1-8$
 $y = \text{unknown}$

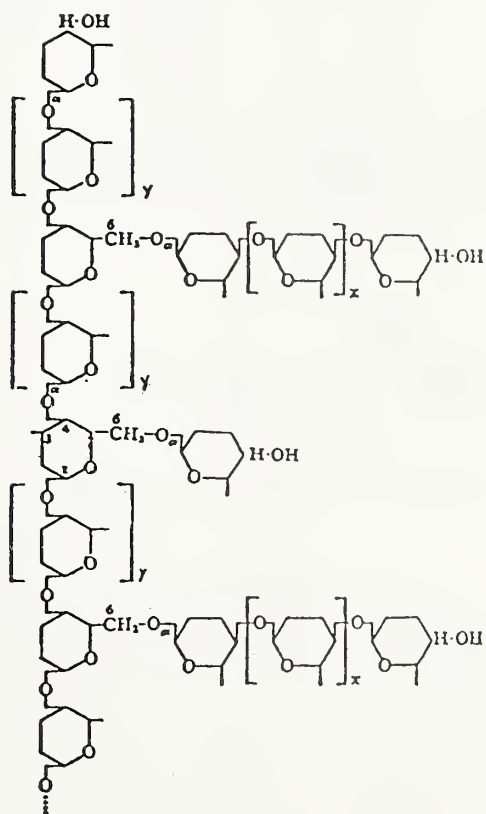
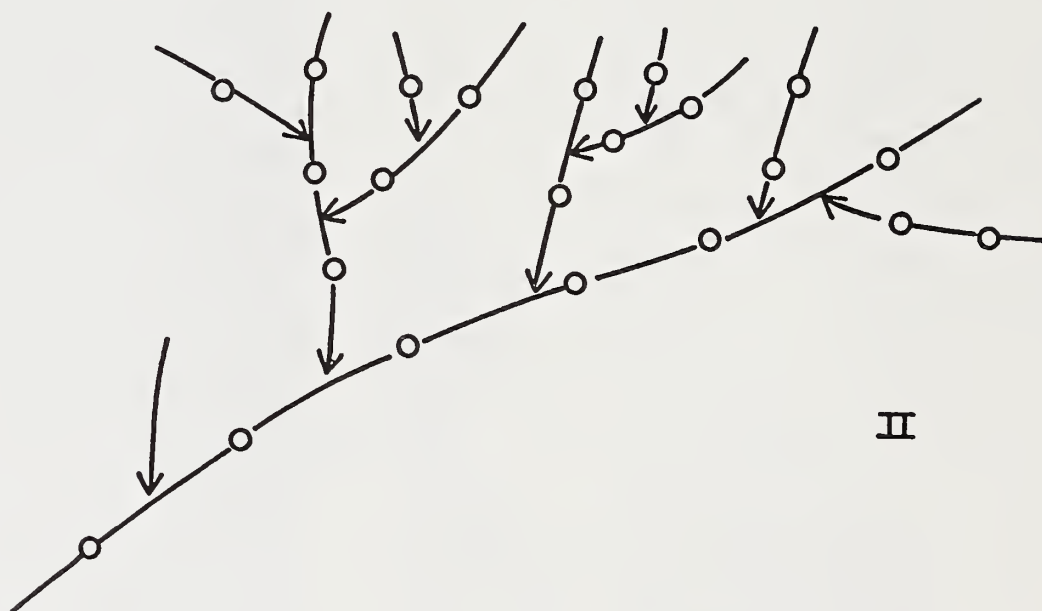
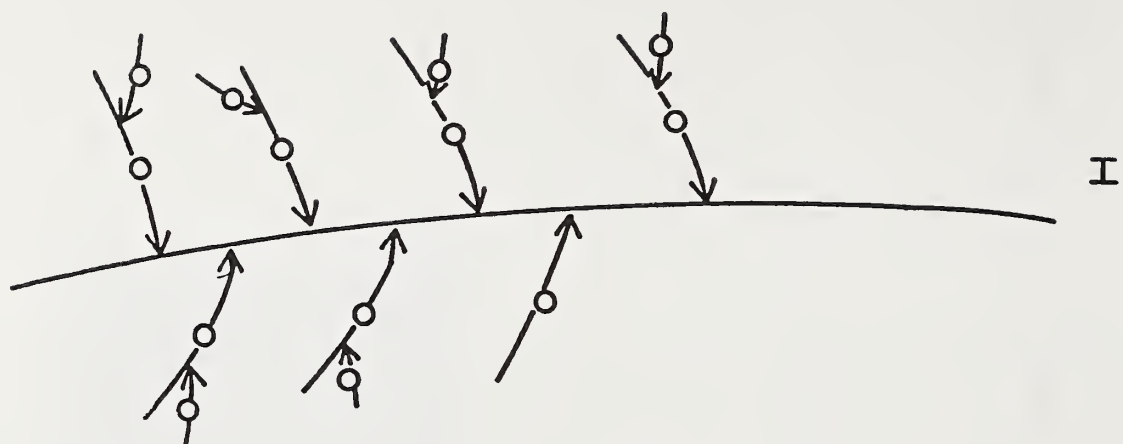


Figure 2.--Proposed structure of sugarcane glucan.



\rightarrow = α -1-6 Linkages

O = α -1-4 Linkages

Figure 3.--Possible structures for sugarcane glucan.

Treatment of the glucan with the debranching enzymes pullulanase and isomaltose yielded maltooligosaccharides ranging from glucose to maltooctaose. Treatment of the material not hydrolyzed by pullulanase with α -amylase yielded only glucose.

Based upon these results, it is concluded that the glucan is a straight chain α -(1-4) linked glucan with α -(1-4) linked side chains ranging in length from glucose to maltooctaose linked to the straight chain by α -(1-6) linkages. The fine structure of the side chain pattern is not yet established.

This structure is similar to amylopectin and glycogen but of lower molecular weight, and with a greater degree of branching. This glucan differs from amylopectin and glycogen in that it is readily soluble in water, giving a clear solution. It may be considered to be a phytoglycogen. It does not show the Maltese cross character of amylopectin under polarized light. The glucan does not appear to have deleterious effects on processing: it does not increase viscosity appreciably, and does not cause acid beverage floc. It does have a high positive polarization which will affect sucrose values of cane juice determined by polarization.

Further work on the characterization of this glucan and its effect on sugar processing is in progress.

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The authors wish to acknowledge the kind assistance and helpful advice of Dr. C.W.S. Tsang, in performing all the HPLC analysis, and of James Harris, in making GC analysis of methylated compounds.

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DISCUSSION

Robert Johnson, BSES: I wonder if you could tell us how the reaction of this glucan with iodine compares with that of starch.

Roberts: The glucan makes a color with iodine but it's a different color from the starch-iodine complex. It makes a reddish-purple or wine color instead of the typical blue color with starch. The color is similar to that made with amylopectin.

Pamela Morel du Boil, SMRI: Does this glucan react positively in the copper-dextran test?

Roberts: No, it does not form a precipitate with the alkaline copper reagent.

Morel du Boil: We found that sarkaran tends to give some response with copper.

Margaret A. Clarke, SPRI: As Earl has mentioned, we do not think that this polysaccharide is sarkaran. Perhaps Earl would like to comment on the possibility that this glucan might be a phytoglycogen, a storage polysaccharide as is found in animals and in some plants, e.g. corn?

Roberts: Yes, glycogen is usually considered animal starch. It is a high molecular weight, very highly branched glucan similar to amylopectin, but several plants are known to have a glycogen-like polysaccharide. Corn, for instance, contains glycogen, called phytoglycogen. We isolated some of that from corn. It had a milky, fluorescent appearance as does glycogen extracted from animals. The cane material formed a completely soluble and clear solution in water. This new polysaccharide is very soluble in water. It is possible that it may still be a type of phytoglycogen.

PERFORMANCE CHARACTERISTICS OF THE CTI® APPARENT PURITY LABORATORY ANALYSIS SYSTEM

James Kysilka and Stanley E. Bichsel
American Crystal Sugar Co.

INTRODUCTION

The recent development of the CTI-501 laboratory Dark Solution Polarimeter (DSP), (Kysilka et al 1983b) and the CTI-601 laboratory flow through reflection refractometer, (Kysilka et al 1983a), coupled with the CTI-1001 Tek Mate microprocessor enables the analyst to determine the apparent purity of high color optically clear process samples directly with a high degree of precision.

The purity analysis system consists of two optical systems --a refractometer to determine total refractometer dissolved solids (RDS) and a polarimeter to determine % sugar.

DISCUSSION OF THE CTI 501 DSP POLARIMETER

Conventional saccharimeters and polarimeters, utilizing light sources of 546 nm or 589 nm, are used in all sugar factories throughout the world to measure the optical rotation of sugar solutions. These conventional instruments are excellent for performing polarization rotation measurements on low colored optically clear sugar solutions in the laboratory, but are inadequate for making precise measurements of highly colored solutions in the laboratory and most on-line process stream monitoring or control applications. Dark solutions are generally decolorized and clarified using lead subacetate solution or dry lead. The use of lead poses problems with respect to hazardous waste disposal, reagent cost per sample, and the so-called lead error effect on pol values.

® CTI registered trademark Crystal Tek International, a
division of American Crystal Sugar Company, Moorhead, MN

The CTI-501 DSP polarimeter has the following technical characteristics:

1. The 875 nm light source permits polarization rotation measurements of highly colored optically clear sugar factory stream juices without the use of lead subacetate clarification.
2. The use of an efficient relatively cold light source and remotely located power supply prevents sample heating and subsequent error.
3. Optoelectronically operated (no moving parts to wear, change position, or slow response time).
4. The operating principle permits a wide range of polarization rotation measurements and is not a limiting factor in the instrument's resolution.
5. Optics and electronic circuitry are sealed for protection against fumes, moisture, and dust.
6. The instrument's versatile CTI-1001 Tek Mate 16 bit microcomputer permits easy adaptability to on-line monitoring, automated laboratory use, and purity measurements when used in conjunction with a refractometer such as the CTI-601 Reflection Refractometer.

TECHNICAL DESIGN DESCRIPTION

An Infrared Emitting Diode (IRED) is used as a light source for the instrument (Figure 1, Item 1). The 875 nm light is collimated by antireflection coated lenses (Item 2). The collimated beam passes through an optional bandpass filter (Item 3) and then through a polarizer (Item 4). The polarizer is present such that the plane of polarization of the beam leaving a midrange sample (50° International Sugar Scale) is at a 45° angle to the splitting surface of the analyzer (Item 6). This 45° beam can be conveniently thought of as composed of two superimposed rays of linearly polarized light having equal intensities. One of these rays is polarized with its electric field vector parallel to the plane of incidence at the analyzer splitting surface, and is called the "P" polarized ray. The other ray is polarized with its electric field vector orthogonal to the plane of incidence, and is called the "S" polarized ray.

The polarized light leaving the polarizer passes through the sample (Item 5) where its plane of polarization is rotated proportional to the amount of optically active material in the sample. The S and P rays are separated by the analyzer (Item 6) and directed to photodiodes (Item 7) for intensity determination. The S and P photodiode output signals are

CTI-501

DARK SOLUTION POLARIMETER

Patent Pending

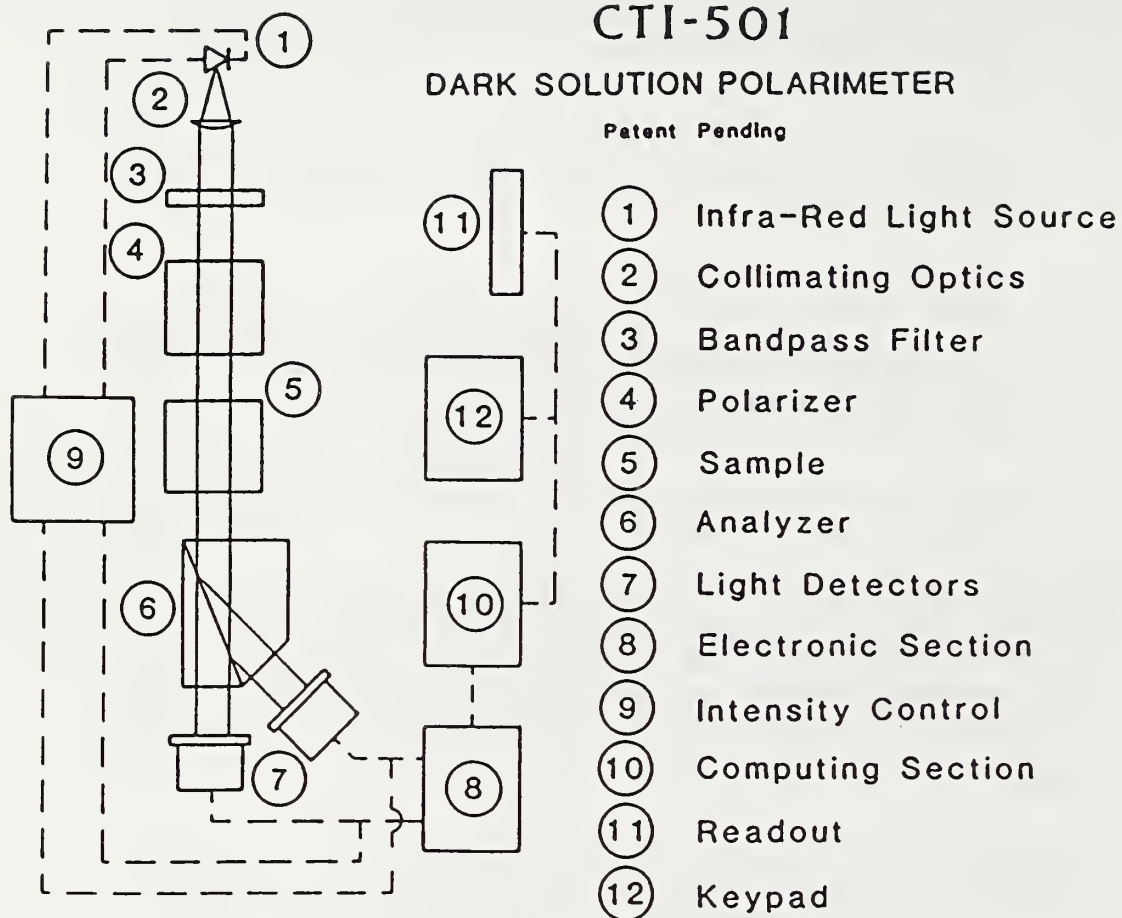


Figure 1.--Diagram of CTI-501 Dark Solution Polarimeter.

amplified by the analog electronics (Item 8) and transmitted to the computer (Item 10). The S and P amplified signals are also transmitted to the IRED intensity control (Item 9) which provides stability to the instrument and automatically increases the IRED light output for dark samples.

If the beam's plane of polarization is rotated 45° either side of its 45° position, the amplitude of the S and P rays would change with $\sin^2 \theta$ and $\cos^2 \theta$ functions as indicated graphically in Figure 2. We have chosen to set the midpoint of our operating range at 45° which fixes the minimum and maximum ends of the operating range approximately 8° either side of the 45° point. The computer through its 16 Bit Analog to Digital (A/D) converters, monitors the amplitude of the S and P rays through the operating range of the instrument. The angle of rotation θ is found using the formula

$$\tan^2 \theta = \frac{\sin^2 \theta}{\cos^2 \theta}$$

when the S ray amplitude = $\sin^2 \theta$ and the P ray amplitude = $\cos^2 \theta$.

The instrument is calibrated by measuring a series of known optically active samples across the range of the instrument and allowing the computer to interpolate a smooth curve through all points.

DESIGN AND OPERATION OF THE CTI-501 DSP POLARIMETER

The CTI-501 Dark Solution Polarimeter is constructed into two separate units. Unit one contains the optical components, optoelectronic components, sample compartment, and analog electronics. Unit two, the computer section, consists of the power supply, computation electronics, digital readout, and keyboard.

The optics unit is housed in an anodized aluminum enclosure and measures 5 3/4" x 6 3/4" x 25 1/8". It consists of three separate compartments. The light source compartment contains the high-power Infrared Emitting Diode (IRED), beam alignment hardware, collimating optics and fixed position polarizer. The sample compartment is separated from the light source and detector compartments by anodized aluminum bulkheads with sealed windows. It will accept all standard polarimeter tubes up to an optical path length of 200 mm. The detector compartment consists of a beam splitting Thompson analyzer which separates the S and P components of the polarized beam, two silicone photodetectors that monitor the S and P beams, and the analog electronics.

OPERATING RANGE (Angle of Rotation)

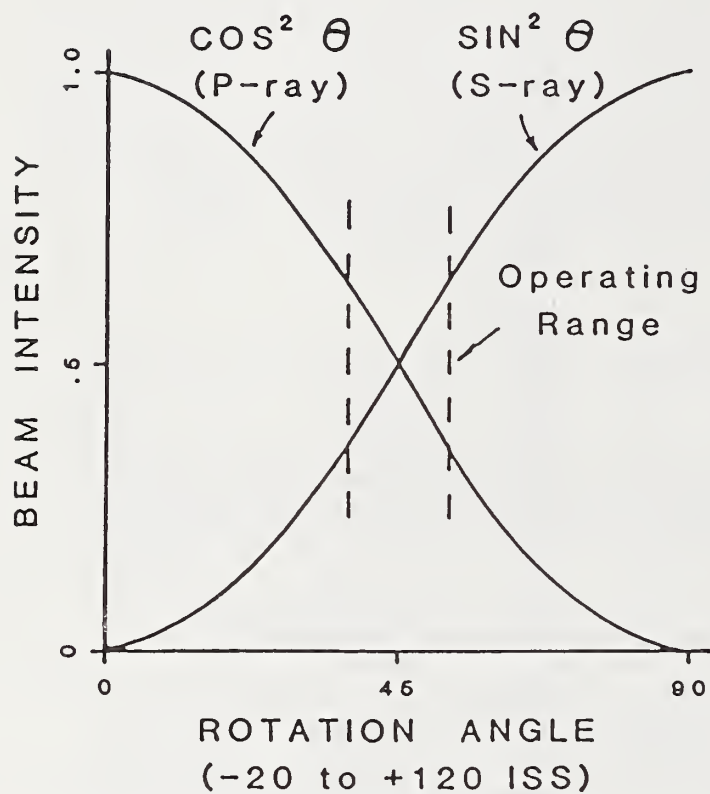


Figure 2.--Operating range of Dark Solution Polarimeter.

The IRED light source is nearly a point source of light, has a life expectancy in excess of 100,000 hours, and emits at 875 nm wavelength. Although the optical power of the 875 nm light beam is similar to most conventional saccharimeters, dark sugar factory stream samples are three orders of magnitude more transparent at 875 nm than at 546 nm (Figure 3). This increased transparency permits the instrument to measure dark sugar factory samples without lead salts clarification.

The computer unit components are mounted on a standard 19" panel for optional console mounting. The computer unit supplies all electrical power for the optics section as well as the computer and display. All computer components including the processor board, memory boards, 16 Bit Analog to Digital (A to D) converter and optional Digital to Analog (D to A) converter board are mounted in a standard buss rack. The 16 Bit microprocessor performs the computation and linearization necessary to convert the photodetector readings into engineering units. These engineering units include International Sugar Scale, percent sugar (on volume), angular degrees as measured at 875 nm, the optical density of the sample at 875 nm. The operating program is stored in Programmable Read Only Memory (PROM); all other information including operator interface information and calibration data is stored in battery backed Random Access Memory (RAM). Operator interface functions include automatic zero, respan, recalibrate, sample cell length, sample dilution (1/2 or 1 normal weight solution), and numerous diagnostic functions. All diagnostic and special operator functions are self-prompting and are presented on the same 20 character alpha numeric display which is used to display sample readout.

The instrument is precalibrated with computed 3rd order least squares curve fit values entered into RAM. Recalibration can be accomplished at any time with a series of four or as many as 10 test samples of known value that cover the range of the instrument. The exact value in International Sugar Scale of quartz test plates at 875 nm is not known, but it is quite close to actual sugar samples. A linear relationship does exist between quartz test plates and sugar. Therefore, calibration of the instrument can be accomplished with quartz test plates and a final span adjustment made with a sugar sample of known value. Automatic zero is accomplished by a single key stroke on the operator interface panel.

Spectrometric Plot of Molasses

WAVE LENGTH Vs TRANSMISSION

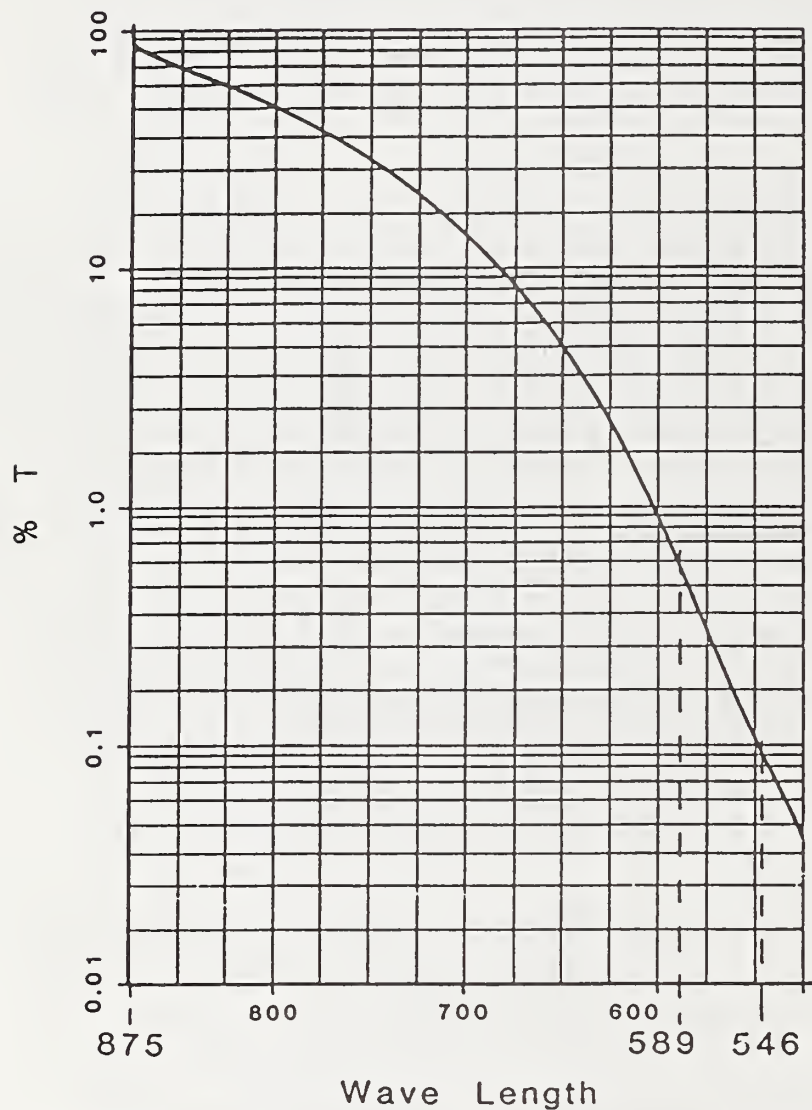


Figure 3.--Spectrometric plot of molasses as a function of wave length vs. transmission.

CIT-501 DSP POLARIMETER RESULTS AND DISCUSSION

The first instrument constructed has been tested under controlled temperature conditions (20°C) along with two other commercially available electronic saccharimeters utilizing a quartz wedge and rotated analyzer respectively. Ninety-three optically clear sucrose solution samples ranging from 0.00° to 100.00° ISS were tested in the three instruments. Results of the tests indicate that at a 99% confidence level there was no significant difference between the CIT-501 Dark Solution Polarimeter and the two commercially available instruments used for comparative evaluation. The mean of the difference between instruments was less than $\pm 0.01^\circ$ ISS units. Seven quartz test plates ranging from -10.00° to 100.00° were read in the three instruments. One of the conventional instruments read the 100.00° plate .02° low and the Dark Solution Polarimeter read the -10.00° plate .02° low. All other readings were within .01° of the calibrated plate value. Five more instruments have now been constructed and have been further evaluated under factory laboratory conditions for a one year period. We have been able to read all optically clear beet sugar factory juice samples directly on the instrument without lead subacetate clarification.

Table 1 compares the accuracy and precision of four pure sucrose solution read six times randomly on the CIT-501 and two Rudolph automatic polarimeters. all polarimeters were calibrated using the same quartz plates. There is no significant difference between the three polarimeters at the 99% confidence level.

Table 1.--DSP comparisons on pure sucrose solutions

	CIT	Rudolph-1	Rudolph-2
	32.45	32.41	32.46
	50.58	50.52	50.56
	60.83	60.71	60.79
	100.11	99.98	100.07
Pooled Std. Error	0.0027	0.0019	0.0021

Each sample read six times in random order on each instrument

DISCUSSION OF THE CTI-601 REFLECTION REFRACTOMETER

Conventional reflection refractometers use the critical angle method of measurement. Reflection refractometers measure the reflected light from the surface of a prism of known refractive index that is in contact with a liquid to be measured. This permits either transparent or highly colored liquids to be measured. Critical angle refractometers usually focus a beam of light on the surface of a prism and/or scan the beam or detector system mechanically in an attempt to locate the critical angle. The critical angle can then be related to the refractive index of the liquid. These focusing and mechanical scanning techniques present numerous possibilities for malfunction or error in the measurement.

This paper describes the CTI-601 reflection refractometer that has the following features:

1. Optoelectronically operated (no moving parts to wear or change position).
2. The light beam is collimated and covers a large area of the liquid/prism interface.
3. The use of an efficient light source and remotely located power supply prevents sample heating and subsequent error.
4. The sapphire measuring prism is resistant to abrasives.
5. Optics and electronic circuitry are sealed for protection against fumes, moisture, and dust.
6. The operating principle permits a wide range of refractive index measurements and is not a limiting factor in the instrument's resolution.
7. The instrument's versatile microcomputer permits easy adaptability to on-line monitoring, automated laboratory use, and purity measurements when used in conjunction with a saccharimeter such as the CTI-501 Dark Solution Polarimeter.

TECHNICAL DESIGN DESCRIPTION

A Light Emitting Diode (LED) is used as a light source for the instrument (Figure 4, Item 1). The light output is collimated by antireflection coated lenses (Item 2). The collimated beam passes through a narrow band filter (Item 3) and then through a polarizer (Item 4). The plane of polarization of the beam leaving the polarizer is 45° to the plane of incidence of the prism surface (Item 6). This 45° beam can be conveniently thought of as composed of two superimposed rays of linearly

CTI-601

REFLECTION REFRACTOMETER

Patent Pending

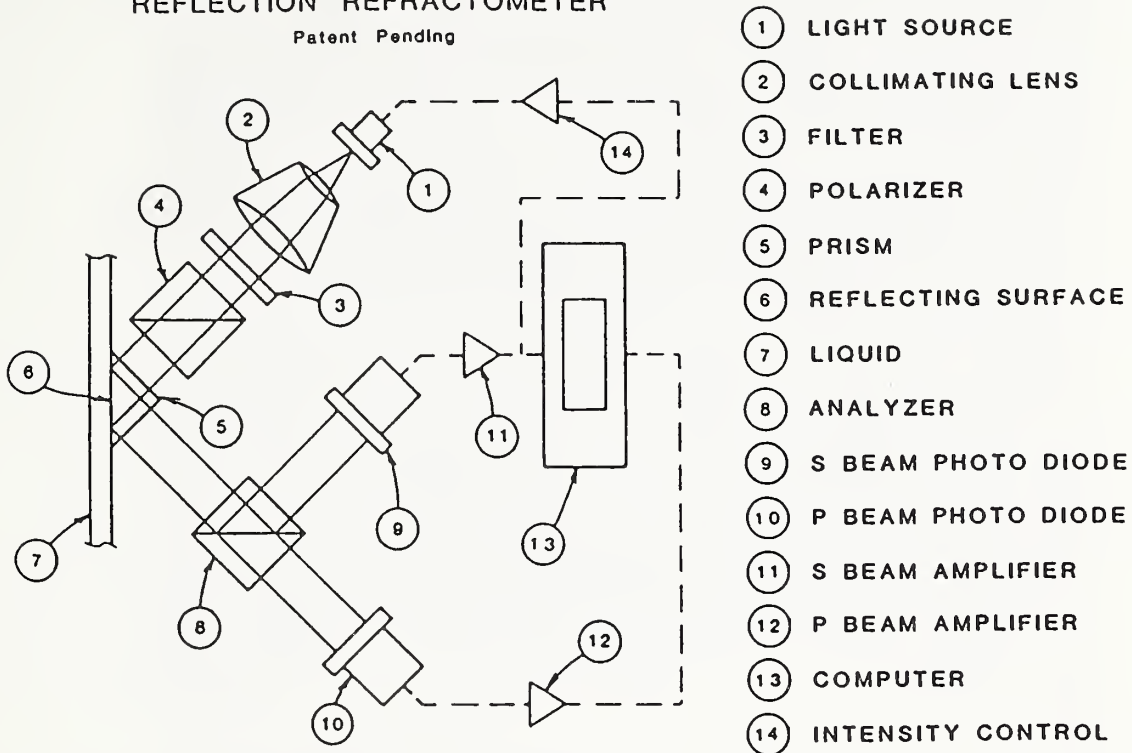


Figure 4.--Diagram of light emitting diode used as light source for Dark Solution Polarimeter.

polarized light having equal intensities. One of these rays is polarized with its electric field vector parallel to the plane of incidence, and is called the "P" polarized ray. The other ray is polarized with its electric field vector orthogonal to the plane of incidence, and is called the "S" polarized ray. The beam of light enters the sapphire prism (Item 5), has a refractive index N^1 . The beam strikes the reflecting surface of the prism/liquid interface (Item 6) at an angle θ^1 . θ^1 is present to a position where the working range of the instrument is less than critical angle θ^C . Part of the light is refracted into the liquid (Item 7) at angle θ and is lost; therefore, θ is not measured directly.

The part of the beam not refracted into the liquid is reflected from the sapphire liquid interface and passes through the analyzer (Item 8) where the S and P polarized rays are separated and directed to photodiodes (Item 9) and (Item 10) for intensity determination. The photodiode output is amplified by amplifiers (Item 11) and (Item 12) and transmitted to a microcomputer (Item 13). In addition the S beam amplified signal is transmitted to the LED intensity control (Item 14) which assures a constant amount of light to the S beam photodiode. The feedback control provides stability to the instrument and corrects for possible reading error due to buildup of light absorbing materials on the optical surfaces.

The microcomputer, in which are stored the values for the prism refractive index n^1 and the angle of incidence, θ^1 , computes the ratio of S and P values $S/P = \text{ratio } (R)$. The angle of the refracted light θ is found using the formula

$$\tan \theta = \frac{1}{\tan \theta^1} \times \frac{R + 1}{R - 1}$$

The refractive index of the liquid being measured, N , is found using the formula

$$N = \frac{N^1 \sin \theta^1}{\sin \theta}$$

The instrument is calibrated by measuring a series of samples of known refractive index across the range of the instrument and allowing the computer to interpolate a smooth curve through all points. The computer also contains software for zero adjustment, span adjustment, and conversion to other engineering units.

DESIGN AND OPERATION OF THE CTI-601 REFLECTION REFRACTOMETER

The CTI-601 Reflection Refractometer is constructed as two separate units. Unit one contains the optical components, optoelectronic components, sample compartment, and analog electronics. Unit two, the computer section, consists of the power supply computation electronics, digital readout, and keyboard.

The optics unit is housed in an anodized aluminum enclosure with a flow through sample compartment. The sample compartment has a removable cover plate for ease of prism cleaning and a heat exchanger for sample temperature control. The unit will function in either a vertical or horizontal position as the application requires.

The LED source emits at 875 nm wavelength, has a life expectancy in excess of 100,000 hours. The sapphire prism is mounted and sealed on a 3/8" anodized aluminum or 316 stainless steel bulkhead that separates the sample area from the optics. The use of a highly efficient LED light source and remote mounting of the power supply minimizes sample compartment heating. A shielded cable of optional length connects the optics section with CTI-1001 Tek Mate microprocessor.

The microprocessor unit components are mounted on a standard 19" panel for optional console mounting. The microprocessor unit supplies all electrical power for the optics unit as well as the digital display. All microprocessor components including the processor board, memory boards, 16 Bit analog to digital (A/D) converter, 12 Bit A/D converter, and optional digital to analog converter boards are mounted in a standard buss rack. The 16 Bit microprocessor utilized in the CTI-1001 Tek Mate performs the computation and linearization necessary to convert the photodetector readings into refractive index or other engineering units. The units include refractive index, Brix, and sample temperature (with other units such as °Baume' optional). The operating program is stored in Programmable Read Only Memory (PROM); all other information including operator interface information and calibration data is stored in battery backed Random Access Memory (RAM). Operator interface functions include zero, respan, recalibrate, and numerous diagnostic functions. All diagnostic and special operator functions are self-prompting and are presented on the same 20 character alpha numeric display which is used to display sample readout.

The instrument is recalibrated with computed 4th order least squares curve fit values entered into RAM. Recalibration can be accomplished at any time with a series of test samples of known refractive index that cover the range of the instrument. Zero or respan is accomplished by entering in the value of a known refractive index sample that is in the sample compartment.

CTI-601 REFLECTION REFRACTOMETER RESULTS AND DISCUSSION

The experimental unit has been tested extensively in the Research and factory laboratories. Preliminary results indicate a precision less than .01 Brix over a range of 0 to 30 RDS at a controlled temperature of 20° C.

Results from experimental data indicate that the algorithm used to linearize the input signals works very well although an offset of 5% between theoretical and experimental data exists. The possibility may exist that values for Θ^1 or N^1 which are preprogrammed into the computer are not correct and the analyzer is not separating the S and P rays completely. This problem is presently being investigated. In any case, the least squares curve fit program does correct for this discrepancy as the refractive index and S/P ratio relationship is linear through the range of the instrument (Figure 5).

Table 2 compares the precision and accuracy of repetitive normal weight molasses determination of refractometer dissolved solids determined using the B&L precision laboratory refractometer and the CTI-601 reflectance refractometer compared with the Karl Fischer method of moisture determination as a reference standard (Rearick et al 1976). There was no significant difference at the 99% confidence level between the CTI-601 reflectance refractometer and the B&L precision refractometer. The B&L precision refractometer gave significantly different results than the Karl Fischer wt. % method at the 95% confidence level. Differences in refractometer dissolved solids measurements due to molasses nonsugar constituents with respect to amount, and specific nonsugars were not found to be significantly different when comparing the CTI-601 and B&L refractometer results on three molasses samples from different factories and at three different normal weight dilutions.

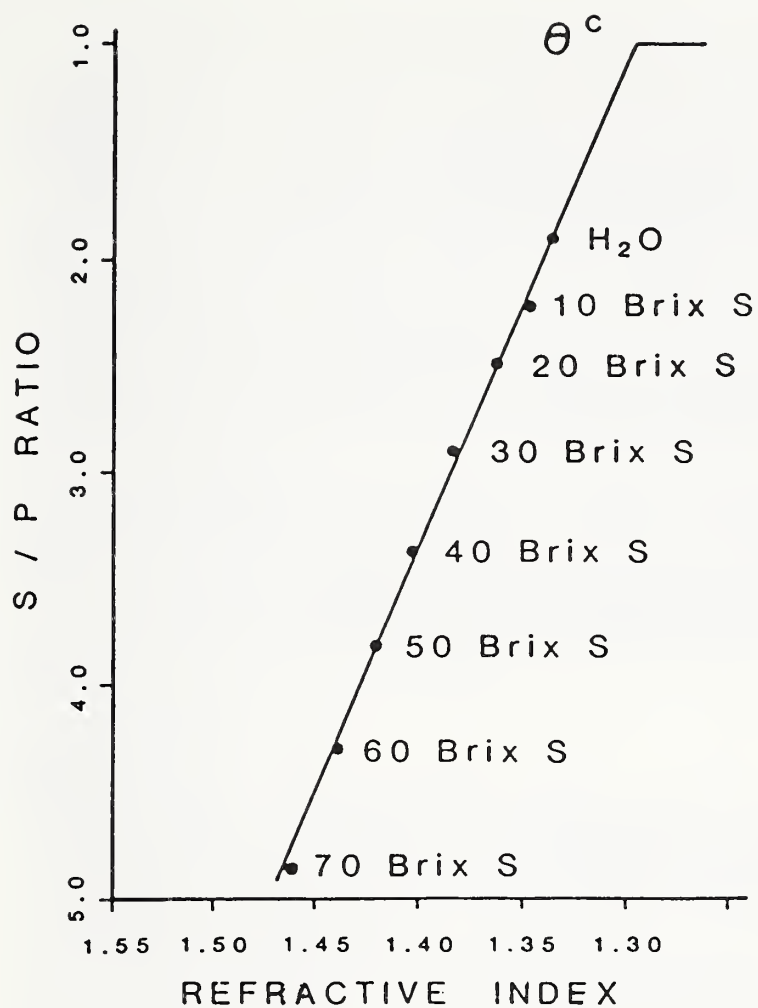


Figure 5.--Least squares curve fit for refractive index vs. S/P ratio in working range of the Dark Solution Polarimeter.

Table 2.--CTI and B&L RDS measurements versus Karl Fischer Wt. % solids measurements

Sample	Molasses		RDS		Karl Fischer Solids Wt. %	Δ RDS (CTI-B&L)	Δ Solids (CTI-KF)	Δ Solids (B&L-KF)
	Dilution		B&L	CTI				
1	1		21.39	21.41	22.13	0.02	-0.72	-0.74
1	2		21.42	21.42	21.66	0.00	-0.24	-0.24
1	3		21.93	21.87	22.91	-0.06	-1.04	-0.98
2	1		20.15	20.07	21.14	-0.08	-1.07	-0.99
2	2		20.18	20.08	21.04	-0.10	-0.96	-0.86
2	3		20.21	20.06	20.37	-0.15	-0.31	-0.15
3	1		20.93	21.09	21.82	0.16	-0.73	-0.89
3	2		21.24	21.15	20.43	-0.09	-0.72	0.81
3	3		21.27	21.15	20.62	-0.12	0.53	0.65
Mean						0.047	0.424	0.778
SD						0.094	0.664	0.698
(Testing the differences in the means)						t = 1.50	t = 1.92	t = 3.34
						$t_{(.975)(8)} = 2.31$		

The B&L refractometer gave significantly different values than the Karl Fischer titration in a paired t test at 95% level. CTI and B&L refractometers agree with each other (F-test at 99% confidence level) better, however, than either agree with titration data. Significant differences due to molasses were not found between CTI and B&L RDS readings using Analysis of Variance (AOV) methods.

SUMMARY

The performance characteristics of the two optical measurement components of the CTI apparent purity analysis system were compared with a standard automatic polarimeter and the Karl Fischer method used as a standard of reference. Precision and accuracy of the optical measurement components were not significantly different at the 99% confidence level. The new system offers the advantage of greater flexibility due to programmable functions, elimination of hazardous waste lead filtrate disposal costs, elimination of the so-called "lead error" and elimination of lead reagent costs. The CTI apparent purity analysis system is easily interfaced with a standard hard copy printer or CRT screen to facilitate automatic laboratory data acquisition.

The development and utilization of an 875 nm light source for measurement of polarization measurements in highly colored optically clear process stream samples establishes the rationale for continuous measurement of % sugar. The CTI polarimeter in conjunction with a flow through refractometer can be utilized to determine apparent purity on a continuous basis.

ACKNOWLEDGEMENTS

We would like to express our appreciation for the many helpful discussions with Bill Barr; the electrical engineering support of Robin Brophy; and the design and test work of Erling Bjorndahl, Tim Haakenson, Jeff Moritz, Steve Ness, and Paul Thompson. In addition, we would like to thank colleagues from the cane and beet sugar segments who have evaluated CTI instrumentation and given us valuable feedback on instrument performance and areas where improvements should be accomplished.

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APPENDIX I

CRYSTAL TEK INTERNATIONAL

A Division of American Crystal Sugar Company

CTI-501 'DARK SOLUTION POLARIMETER GENERAL SPECIFICATIONS

CTI-501 Dark Solution Polarimeter - This laboratory instrument has the capability of reading measurements of all optically active solutions including optically clear normal weight sugar solutions and/or molasses syrups, without using clarification agents such as lead salts. Key operational features of the CTI polarimeter include fast response time, self-prompting operation, no moving parts, versatility, and ease of calibration. The CTI-501 uses the CTI-1001 Tek Mate. This same intelligent console can be used in conjunction with the CTI-601 Refractometer for Brix and purity determinations of sugar solutions and/or molasses syrups.

SPECIFICATIONS

General

Model	CTI-501
Scale	°S (ISS) other scales and ranges
Range	-20°S to 120°S programmable or optional
Resolution	.01°S
Accuracy	.02°S clear solutions .04°S dark solutions
Response Time	5 seconds
Power Requirements	115 Volts 50-60 Hz other voltages optional

Optics Section

Material	3/8" anodized aluminum housing
Weight	37 pounds
Size	5-3/4" x 6-3/4" x 25 1/8"
Mounting	Horizontal or vertical
Light Source	Higher power infra-red emitting diode Average life in excess of 20,000 hours
Wave Length	875 nm
Detectors	Planar diffused silicon photodiodes
Sample Compartment	Accepts standard test plates and sample tubes with 30 mm diameter ends to 100 mm long.

Computation Section (CTI-1001 Tek Mate)

Type	Microcomputer based
Inputs	16 bit A/D
Outputs	20 character alpha numeric
Operation	Automatic
Re Zero	Pushbutton - automatic
Operator Interface	Pushbutton with self-prompting thru display
Size	9" x 9-1/2" x 12"

Other Functions

Re Span	Permits calibration slope change when instrument is operated at room temperatures other than 20°C.
Re Calibration	Permits complete recalibration of instrument.
Cell Length	Corrects reading for different cell lengths.
Concentration	Choice of operating instrument with 1.0 normal or 1/2 normal weight solutions.
% Sugar	Expressed as wt/vol and assumes pure sucrose solutions.
Optical Absorbance	Uncalibrated value

Numerous other evaluation functions.

APPENDIX II

CRYSTAL TEK INTERNATIONAL

A Division of American Crystal Sugar Company

CTI-601 REFRACTOMETER GENERAL SPECIFICATIONS

CTI-601 Refractometer - This is a precision instrument for laboratory and on-line applications. It has many features such as no moving parts, automatic operation, temperature corrected readings, and a wide refractive index range. Its microcomputer permits the refractive index reading to be converted into many other engineering units. The CTI-601 uses the CTI-1001 Tek Mate. This same intelligent console can be used in conjunction with the CTI-501 Dark Solution Polarimeter for determination of sugar pol (percent sugar) for sugar solutions and/or molasses syrup.

SPECIFICATIONS

General

Model	CTI-601
Scale	Brix (other scales and ranges programmable or optional)
Resolution	.00001 R.I.
Accuracy	.00005 R.I.
Response Time	5 seconds
Power Requirements	115 volts 50-60 Hz other voltages optional

Optics Section

Material	3/8" anodized aluminum housing
Weight	10.5 pounds
Size	7" x 8" x 7 1/2"
Mounting	Horizontal or vertical
Light Source	Light emitting diode
Wave Length	875 nm
Detectors	Planar diffused silicon photodiodes
Sample Compartment	Flow thru or grab sample

Computer Section (CTI-1001 Tek Mate)

Type	Microcomputer based
Inputs	16 bit A/D
Outputs	20 character alpha numeric
Operation	Automatic
Re Zero	Pushbutton - automatic
Operator Interface	Pushbutton with self-prompting thru display
Size	9" x 9-1/2" x 12"
Weight	14.5 lbs.

Other Functions

Temperature Readout	Displays sample temperature
Re Span	Permits calibration slope change
Re Calibration	Permits complete calibration of instrument

Numerous other evaluation functions.

DISCUSSION

Randolph R. Tamaye, HSPA: Has this instrument made any difference in your pol or sucrose balance across your house, considering the fact that you aren't clarifying with any lead solutions?

Bichsel: We don't see any significant difference, but let me say this. In our factory operations, we have a loss called the unknown loss. As you know, that's the difference figure that makes the balance perfect. I'm afraid that in our particular case, the unknown loss probably precludes our picking up the small difference. There is too much static due to the unknown loss to pick up a clear signal.

Tamaye: On your lower purity streams, did you notice any difference between the apparent pol values with and without the lead clarification?

Bichsel: We think we do see some differences, some significant differences, particularly in our molasses.

Tamaye: Are you using this polarimeter to analyze the incoming raws without lead clarification?

Bichsel: We have not done this. I think that some other people will do it. We are in the beet sugar business so we don't have any incoming raws.

John Lopez-Ona, Colonial Sugars: The way this session started today, there were two very heavy statistical presentations by Dr. Williams and Mary An Godshall. In the area of comparing polarimeters, I presented a paper at the SIT in 1977, also very heavy in statistics. Now, my question is--did you compare several polarimeters against the CTI? We did this between the Schmidt & Haensch and the Rudolph. Did you use any statistical approach to compare the averages of the CTI against the Rudolph or the Schmidt & Haensch?

Bichsel: Yes, we did. It's covered in the paper. There were several hundred paired samples. I didn't present all that data here, but we were able to determine confidence levels using the same quartz plates to standardize the Schmidt & Haensch, the CTI, and the Rudolph automatic polarimeter. There was no difference. Incidentally, we used your paper as a guide in setting up our data.

Stanley E. George, B.C. Sugars: Are these units available to be seen? Are visitors welcome to come to the factory and see it?

Bichsel: Yes, in fact, we will have it on display tomorrow.

CHANGES IN JUICE COMPOSITION OF SUGARCANE AS AFFECTED BY POST-FREEZE DETERIORATION IN LOUISIANA

Benjamin L. Legendre, USDA-ARS, U.S. Sugarcane Field Laboratory, and W.S. Charles Tsang and Margaret A. Clarke, Sugar Processing Research, Inc.

INTRODUCTION

In general, sugarcane (Saccharum interspecific hybrids) damaged by a severe freeze produces juices of lowered purity and sucrose and increased titratable acidity, and an abnormally high amount of dextrans and gums (Coleman 1952, Friloux et al. 1965, Gascho and Miller 1979, Irvine 1972, Irvine and Friloux 1965, Miller and Gascho 1975). As early as 1938, Fort and Lauritzen (1938b, 1939) reported that an increased gum (polysaccharide) content accompanied the formation of excess acidity in the juice of frozen sugarcane. Later, Lauritzen et al. (1949) related changes in juice composition to the degree of freeze injury. The greater the extent of injury (number of buds killed), the more rapid the increase in gums with a simultaneous decrease in Brix, sucrose and purity and an increase in acidity.

Following freeze injury, dead and moribund cells become vulnerable to invasion by the bacterium, Leuconostoc mesenteroides. This bacterium, which is ubiquitous in cane fields, utilizes sucrose and produces dextran as a by-product (Irvine and Legendre 1985). The bacterium's entry into storage tissue is facilitated by dead lateral buds (-4.4°C or 24°F) and by freeze cracks (-5.6°C or 22°F). An increase in the level of dextran is not the only symptom of Leuconostoc involvement in post-freeze deterioration. As the organism consumes sucrose, there is a release of fructose along with a lowered pH of the juice (Imrie and Tilbury 1972). Further, Irvine and Legendre (1985) proposed two mechanisms for deterioration: susceptibility of tissue to freezing and susceptibility to the formation of dextran and/or polysaccharide after the freeze. In these studies, the commercial variety CP 70-321 had been identified as among the most resistant to deterioration following freezing, whereas, L 65-69 was among the least. CP 72-370 was found intermediate in deterioration following freezing.

Christmas day, 1983, brought a record daily low temperature for December, -10.6°C (13°F) at Houma, LA, as farmers were harvesting the last of their crop. Following the freeze, the weather remained cool throughout the 15-day sampling period with an average temperature of 4.9°C (41°F). When the sugarcane stalks and soil began to thaw on the afternoon of December 26, it became apparent that all stalk tissue in all varieties was frozen to ground level. Tops began to fall several days later, and during the second week after the freeze the stalks of some varieties bent near ground level producing a lodging effect not usually seen in cane. The post-freeze period was the first opportunity for field evaluation, after a freeze of this magnitude, of the deterioration of several sugarcane varieties grown predominately in Louisiana.

The objectives of this study were to determine the changes in juice composition of sugarcane as affected by post-freeze deterioration, to compare methods of analyses and to determine the associations among juice components as they relate to deterioration.

MATERIALS AND METHODS

Variety trials for estimating cold tolerance in the field are planted annually at Houma, LA. From 8 to 15 varieties are planted in the fall of each year, and they are sampled repeatedly during the winter (the following year). Planting is done on raised ridges 1.8 meters apart; variety plots are 12 m long and 3 rows wide. The experimental design is a randomized block with 4 replications. After planting, the cane is covered with 5 cm of firm soil and, when spring regrowth appears, soil is drawn to the new shoots with repeated cultivations. Weeds are controlled with a standard pre-emergence herbicide, and the cane is fertilized with ammonia at approximately 90 kg N/ha.

The eight varieties planted in the fall of 1982 for estimating cold tolerance were: CP 70-321, CP 72-356, CP 72-370, CP 74-383, CP 76-301, CP 76-331, L 65-69 and NCo 310. All are or have been grown commercially in Louisiana with the exception of CP 76-301. CP 76-301 was a candidate variety for commercial release; however, it was dropped from the testing program in 1984 because of low yields in the stubble crop.

Sampling was begun on December 23 and continued until January 9, 1984. Sampling was confined to the center row of the three-row plot, and 15 stalk samples were taken serially along the row on each sampling date. Stalks were cut at ground level by hand and topped approximately at the position of the terminal bud.

Stalks were crushed once in a 3-roll sample mill, and the expressed juice subsampled after thorough mixing. Laboratory analyses of one set of subsamples were performed by standard methods: Brix by hydrometry; apparent sucrose (as % of juice)

by polarimetry following clarification with lead subacetate; dextran by the haze or CSR method (Hidi et al. 1974, Keniry et al. 1969, Meade and Chen 1977, Nicholson and Horsley 1959); juice pH with a pH meter; and, juice acidity by titration. The latter was done by determining the volume of 0.1N NaOH required to raise the pH of 50 ml of juice to pH 8.3; data are expressed in terms of volume of 0.1N NaOH/10 ml of juice.

Samples of juice from badly deteriorated cane which did not clarify with lead subacetate were clarified by the Herles method (Bates 1942). In this method, juice (52 g) is placed in a sugar flask and 5 ml of an aqueous solution of neutral lead nitrate (500 g/L) is added, followed by 5 ml of an aqueous solution of sodium hydroxide (50 g/L). The sample and reagents are mixed and allowed to settle. If clarification is satisfactory, the sample is diluted to the mark, mixed and filtered; if not, a new sample is weighed, and up to 15 ml of each reagent may be added for each sample. Pol is measured in tubes of appropriate length, and corrections are made for length and dilution.

A second set of subsamples were frozen immediately after milling and transported to Sugar Processing Research, Inc. (SPRI), New Orleans, for a more detailed analysis of 3 of the 8 varieties in the study: CP 70-321, CP 72-370 and L 65-69. Sucrose, glucose and fructose were analyzed by high performance liquid chromatography (HPLC) using a BioRad HPX-87C carbohydrate column (Clarke and Tsang 1983); dextran by Roberts' copper method (Roberts 1983); and, total polysaccharides as the alcohol-insoluble reactants with phenol-sulfuric acid (Roberts and Friloux 1965).

The data for each variable were subjected to analyses of variance and the means separated by Least Significant Difference (L.S.D.) Tests (Steel and Torrie 1960). The data were also analyzed by linear regression and correlation coefficients among variables were computed.

RESULTS AND DISCUSSION

Examination of the large amount of data accumulated over a 10-year period showed that deterioration of sugarcane following freezing is best measured by juice sucrose, titratable acidity and dextran content, and these values are shown in Tables 1, 2, and 3. Cane sampled just before the freeze produced juice of excellent quality with expected significant varietal differences in sucrose and titratable acidity, but not in dextran content. Samples taken at 10 and 12 days after the freeze showed extensive changes in juice composition and, by the 15th day, all varieties were unacceptable for processing. There were even then significant varietal differences in sucrose, titratable acidity and dextran content, just as there were at 10 and 12 days after the freeze. The variety CP 70-321 was relatively superior on all post-freeze sampling dates, having the highest sucrose content and lowest titratable acidity and dextran

Table 1.--Post-freeze changes in apparent sucrose content of eight varieties at Houma, LA.

Variety	Apparent sucrose (%) ¹			
	Days after freeze ²			
	-2	10	12	15
CP 70-321	17.53	15.87	16.26	13.12
CP 72-356	16.51	15.12	13.89	9.06
CP 72-370	17.45	15.47	15.70	11.88
CP 74-383	17.48	15.74	14.90	11.07
CP 76-301	16.16	14.92	11.72	9.85
CP 76-331	18.71	17.10	14.99	10.50
L 65-69	17.01	14.52	10.48	6.34
NCo 310	15.70	15.00	13.58	5.71
LSD .05	1.01	0.87	2.51	4.70

¹Apparent sucrose by polarization.

²Freeze, -10.6°C on December 25, 1983.

Table 2.--Post-freeze changes in titratable acidity of eight varieties at Houma, LA.

Variety	Titratable acidity (ml 0.1N NaOH/10 ml juice)			
	Days after freeze ¹			
	-2	10	12	15
CP 70-321	1.96	2.14	3.20	6.68
CP 72-356	2.54	3.20	5.11	9.70
CP 72-370	2.14	2.82	3.83	7.84
CP 74-383	2.07	2.61	3.95	7.12
CP 76-301	2.11	2.98	4.61	7.24
CP 76-331	2.32	2.73	4.73	8.32
L 65-69	2.38	3.21	5.56	7.79
NCo 310	2.30	2.57	4.57	7.84
LSD .05	0.24	0.32	0.68	1.56

¹Freeze, -10.6°C on December 25, 1983.

Table 3.--Post-freeze changes in dextran content of eight varieties at Houma, LA.

Variety	Dextran content ¹ (mg/ml)			
	Days after freeze ²			
	-2	10	12	15
CP 70-321	0.10	0.51	4.28	8.09
CP 72-356	0.12	1.38	10.10	15.15
CP 72-370	0.11	0.86	5.92	10.58
CP 74-383	0.11	0.79	7.08	11.35
CP 76-301	0.11	1.27	10.66	11.85
CP 76-331	0.11	0.73	6.91	12.68
L 65-69	0.10	1.38	10.15	14.92
NCo 310	0.10	0.73	7.28	14.60
LSD .05	NS	0.49	2.60	4.57

¹Dextran by haze method.

²Freeze, -10.6°C on December 25, 1983.

content. The variety NCo 310 exhibited good keeping qualities on the 10th day but deteriorated badly after that and ended no different from L 65-69.

A second analysis for dextran using Roberts' copper method for three varieties, CP 70-321, CP 72-370 and L 65-69, showed a considerably lower concentration than that found using the haze method (Table 4). In Table 3, CP 70-321 had the lowest concentration of dextran throughout the sampling period and L 65-69 had one of the highest. However, when the concentration of dextran for L 65-69 is measured by the Roberts' copper method, it was lower than that found for CP 70-321 or CP 72-370 (Figure 1). On the initial sampling date in unfrozen cane the two methods gave similar results; however, with time after the freeze the haze method gave dextran concentrations from 3 to 20 times that found by the Roberts' copper method. The reason for this difference is that the haze method is not specific for dextran and the results indicated that other polysaccharides interfered with the method, inflating results (Roberts 1983). The Roberts' copper method for dextran separates all the polysaccharides from the sugar and the dextran is selectively precipitated with alkaline copper sulfate.

An effect of dextran in sugarcane juice is its interference with analytical tests for sucrose and purity (Imrie and Tilbury 1972). Dextran is highly dextrotatory and therefore can inflate

Table 4.--A comparison of analytical methods for the determination of dextran content of three varieties as affected by post-freeze deterioration at Houma, LA

Variety	Method of Analysis ¹	Dextran content (ppm, dry weight X 1000)			
		Days after freeze ²			
		-2	10	12	15
CP 70-321	Haze	0.534	3.017	24.660	58.755
	Roberts'	0.206	0.941	6.837	18.958
CP 72-370	Haze	0.591	5.228	35.500	84.994
	Roberts'	0.771	8.077	15.210	24.226
L 65-69	Haze	0.551	9.004	92.932	231.497
	Roberts'	0.246	14.243	10.547	10.751
LSD .05	Haze	NS	2.396	12.569	22.860
	Roberts'	NS	6.148	6.887	10.045

¹Haze method, samples analyzed at Houma, LA; Roberts' copper method, samples analyzed at Sugar Processing Research, Inc., New Orleans, LA.

²Freeze, -10.6°C on December 25, 1983.

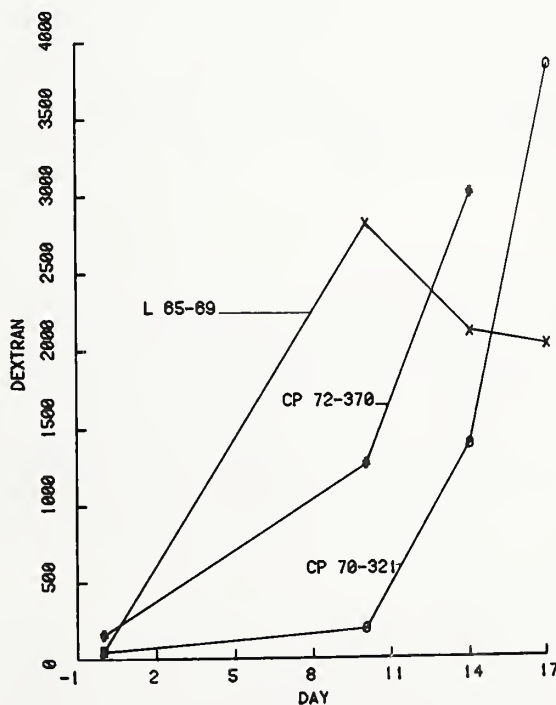


Figure 1.--Dextran (PPM, juice) in frozen cane of three varieties at Houma, LA

the direct polarization reading of juice samples, unless removed prior to analysis. High performance liquid chromatography (HPLC) reads sucrose alone while polarization reads a combination of optically active substances. A comparison of the two analytical methods for the determination of sucrose content is shown in Table 5. Although it was shown in Table 4 that the

Table 5.--A comparison of analytical techniques for the determination of sucrose content of three varieties as affected by post-freeze deterioration at Houma, LA

Variety	Method of Analysis ¹	Sucrose content (%)			
		Days after freeze ²			
		-2	10	12	15
CP 70-321	AS	17.53	15.87	16.26	13.12
	HPLC	18.26	16.87	15.15	12.57
CP 72-370	AS	17.45	15.47	15.70	11.88
	HPLC	17.89	16.18	14.74	11.94
L 65-69	AS	17.01	14.52	10.48	6.34
	HPLC	16.31	14.21	9.66	7.02

¹AS as apparent sucrose by polarization analyzed at Houma, LA; HPLC as high performance liquid chromatography (HPLC) analyzed at Sugar Processing Research, Inc., New Orleans, LA.

²Freeze, -10.6°C on December 25, 1983.

juice had an appreciable amount of dextran, especially with time after the freeze, there were no significant differences in sucrose content between the two analytical procedures. A correlation analysis between apparent sucrose as measured by polarization and sucrose by HPLC for the samples of three varieties, CP 70-321, CP 72-370 and L 65-69, gave a highly significant r value of 0.97. This indicated an almost perfect agreement between the two procedures. Apparently, dextran as well as other polysaccharides, were removed from the juice by the clarifying agent, lead subacetate, where sucrose was measured by polarization.

Concurrent with the increase in dextran concentration that occurs in deteriorating sugarcane juice is an increase in the amount of fructose. According to Imrie and Tilbury (1972) Leuconostoc mesenteroides is a sucrose utilizing organism. Sucrose is converted to dextran by the enzyme dextran-sucrase

leaving fructose as a by-product. Dextran consists of a basic straight-chain polymer of $-(1 \rightarrow 6)$ linked glucose units, with some branches linked by $-(1 \rightarrow 3)$ or $-(1 \rightarrow 4)$ glucoside bonds, leaving most of the fructose behind in solution. A closer look at the composition of sugarcane juice as affected by post-freeze deterioration showed a significant increase in fructose on each date of analysis and for each of the three varieties (Table 6). On the other hand, the glucose content increased only slightly between the pre-freeze sampling date and

Table 6.--Post-freeze changes in invert sugar (glucose and fructose) of three varieties as affected by post-freeze deterioration at Houma, LA

Variety	Invert Sugar (%) ¹									
	Days after freeze ²									
	-2		10		12		15		LSD .05	
	GLU	FRU	GLU	FRU	GLU	FRU	GLU	FRU	GLU	FRU
CP 70-321	0.18	0.19	0.46	0.59	0.51	1.33	0.52	1.74	0.11	0.35
CP 72-370	0.03	0.17	0.35	0.74	0.42	1.68	0.46	2.18	0.12	0.54
L 65-69	0.27	0.31	0.33	1.42	0.25	2.90	0.28	3.43	NS	1.30
LSD .05	0.06	0.02	NS	0.63	0.14	0.80	0.07	1.27	--	--

¹GLU = Glucose; FRU = Fructose. Analyzed by high performance liquid chromatography (HPLC) at Sugar Processing Research, Inc., New Orleans, LA.

²Freeze, -10.6°C on December 25, 1983.

10 days after the freeze for two of the three varieties; thereafter, there was no change in the glucose content of the juice for all three varieties. No change was noted for the variety L 65-69 throughout the sampling period. Total invert sugar (glucose and fructose) was lowest on the pre-freeze harvest date while sucrose was highest; after which, as the concentration of invert sugar (predominately fructose) increased, the concentration of sucrose decreased.

An increase in soluble polysaccharide (gum) of sugarcane juice has been associated with lower quality (Fort and Lauritzen 1938b). Although U.S. Mainland sugarcane producers have long used titratable acidity as a quality indicator when milling frozen cane, Irvine (1972) noted that the levels of titratable acidity may be influenced by location or other factors, and normal cane may have titratable acidity at levels exceeding the

lower permissible penalty levels (Irvine 1964). In the present study there was no statistical difference in total polysaccharide on the pre-freeze sampling date, although, L 65-69 had nearly twice the measurable gums of CP 70-321 (Figure 2). At day 10 and 12, L 65-69 had significantly higher gums than both CP 70-321 and CP 72-370. By day 15, it appeared that gum levels were biased by the presence of protein (yeast, bacteria) which interfered with the measurement. It is interesting to note that while L 65-69 showed the highest initial dextran formation after the freeze, it does not continue to produce dextran but does produce additional gum. On the other hand, the variety CP 72-370, while initially producing less dextran than L 65-69 on day 10 after the freeze, produces more than either CP 70-321 or L 65-69 by day 12 and again on day 15. The variety CP 70-321 is clearly most resistant to deterioration. These data support the findings of Irvine and Legendre (1985).

Dextran content (PPM on juice) was regressed on the other quality indicators; regression coefficients, correlation coefficients and coefficients of determination are shown in Table 8. All regression and correlation coefficients were significantly different from zero at the 1% level of probability. The coefficients of determination (r^2) indicate that two of the independent variables, pH and titratable acidity, are closely associated with an increase in dextran content and therefore could be used to predict that deterioration has occurred. On the other hand, apparent sucrose was not ($r^2 = 0.27$) and

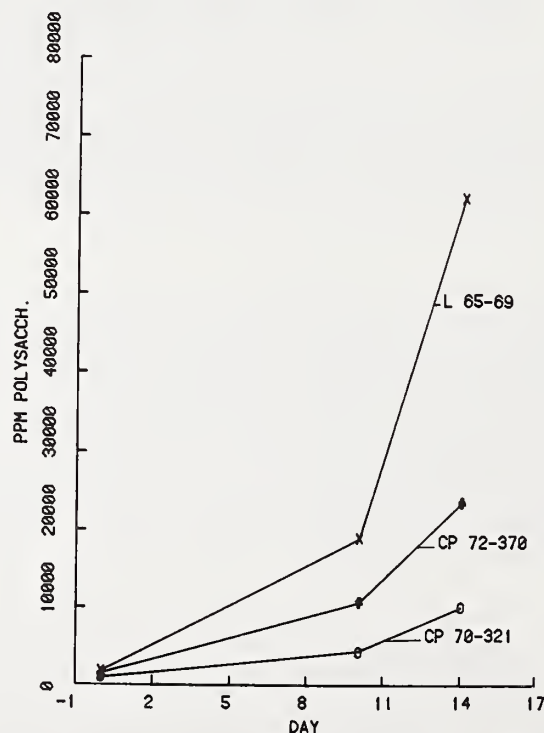


Figure 2.--Total polysaccharides (PPM, dry weight) in frozen cane of three varieties at Houma, LA

therefore should not be used as an indicator of deterioration. These findings are in keeping with the results of Gascho et al. (1973) who reported a highly significant correlation between juice pH and dextran ($r = -0.90^{**}$) when studying sugarcane deterioration during storage. However, they found that dextran measurements appeared slightly more sensitive.

Correlation analyses for pH and apparent sucrose, pH and titratable acidity and titratable acidity and apparent sucrose showed a close interrelationship amongst these three indicators of deterioration (Table 7). The coefficients of determination (r^2) of 0.71, 0.95 and 0.76, respectively, indicated that any one of the three variables was a reasonably good predictor of the other. However, according to Fort and Lauritzen (1938a), the use of excess acidity (titratable acidity) rather than total acidity (pH) is recommended for the evaluation of deteriorated cane.

SUMMARY

The results of this study suggest that when all variety differences due to the resistance of tissue to freezing are removed (as was the case in this study) there are still highly significant differences in the rate of deterioration and these differences can be very important in sugar processing. Since tissue resistance was not a factor in this study, and since varietal differences were still evident, it is possible that resistance to deterioration is related to resistance to the dextran producing microorganism, Leuconostoc mesenteroides.

Deterioration was initially measured by changes in Brix, apparent sucrose, apparent purity, pH, titratable acidity and dextran content of the juice. Significant pre-freeze differences occurred among varieties for Brix, apparent sucrose and purity and titratable acidity, but not for pH and dextran content. Post-freeze varietal differences were sometimes evident for all parameters. A more detailed analysis of juice from three varieties showed that apparent sucrose by polarization and true sucrose by high performance liquid chromatography (HPLC) gave similar results even when the dextran of the juice was excessive. Although there was an apparent relationship in the dextran content of the juice as measured by the haze and Roberts' copper methods, the haze method was non-specific for dextran, giving values from 3 to greater than 20 times higher than the Roberts' copper method in severely deteriorated juice. Results also showed that fructose can be used as a measure of deterioration. While glucose remained essentially constant throughout the sampling period, the level of fructose increased as sucrose was consumed by the apparent action of the Leuconostoc microorganism.

These data suggest a close relationship between post-freeze deterioration of sugarcane varieties and the apparent sucrose,

Table 7.--Regression coefficients, correlation coefficients (r) and coefficients of determination (r^2) for several parameters post-freeze deterioration of three sugarcane varieties¹

y^2	X	a	b	r	r^2
Dextran (ppm, Juice)	Apparent sucrose (%)	5356.86	- 251.70**	-0.52**	0.27
Dextran (ppm, Juice)	pH	13426.25	-2318.65**	-0.80**	0.64
Dextran (ppm, Juice)	Titratable acidity (ml 0.1N NaOH/10 ml juice)	-452.21	571.30**	0.79**	0.62
pH	Apparent sucrose (%)	3.02	0.141**	0.84**	0.71
pH	Titratable acidity (ml 0.1N NaOH/10 ml juice)	5.98	-0.244**	-0.98**	0.95
Titratable acidity (ml 0.1N NaOH/10 ml juice)	Apparent sucrose (%)	12.19	-0.585**	-0.87**	0.76

¹Freeze, -10.6°C on December 25, 1983.

² $y = a + bX$.

**Significant at the 0.01 probability level.

pH, titratable acidity and dextran content of their juices. Juice sucrose and dextran content are important factors in recovery of sugar by the factory, and should be given consideration in selecting varieties for resistance to deterioration following a freeze.

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DISCUSSION

Joseph A. Polack, Audubon Sugar Institute: Ben, you didn't mention what the ambient temperatures were for Day 0 to Day 17. Our experience is that this has a great deal to do with the rate of deterioration.

Legendre: In this study, all varieties were exposed to a natural freeze of -10.6°C on December 25, 1983. Below freezing temperatures actually occurred for 59 hours, beginning the evening of December 24. On December 26, the stalks were still frozen solid in standing field cane. Complete thawing did not occur until December 27 when the high temperature reached 23°C ; however, by the next day, with the passage of a new cold front, daily high temperatures did not exceed 7°C for the next three days with freezing conditions occurring each night until January 3, 1984. After January 3 there was a gradual warming trend. However, the cold temperatures during the first 10 days after the killing freeze helped to reduce the rate of deterioration, especially in the variety CP70-321. Both CP72-370 and L65-69 deteriorated rapidly in spite of the cold temperatures. Beyond 10 days after the freeze, all varieties deteriorated rapidly as the warming trend continued.

Polack: I think the point is: had you had warm weather sooner, the deterioration would have been even faster.

Legendre: Most definitely so.

Alan M. James, Consultant: Just a point of clarification, are the yields expressed in short tons of 2000 pounds?

Legendre: Yes, the data is expressed as yield of theoretical recoverable sugar (in pounds) per short ton of cane.

Robert J. McCowage, CSR Ltd.: I wonder if you could tell me the history of the samples that were used in this trial. Were they mill samples, or how were they obtained?

Legendre: Variety trials for estimating cold tolerance in the field are routinely planted at Houma, LA. Planting is done on raised ridges 1.8 meters apart, variety plots are 12m long and 3 rows wide. The experimental design is a randomized block with 4 replications. Sampling was begun at the beginning of the post-freeze period and confined to the center row of the 3-row plot, and 15 stalk samples were taken serially along the row on each sampling date. Stalks were cut at the ground level by hand and topped at approximately the position of the terminal bud. Stalks were crushed once in a 3-roll sample mill, and the expressed juice subsampled after thorough mixing, the time between cutting and crushing being less than one hour.

McCowage: The Australian experience is that it is very difficult to detect dextran in free-standing cane and in manually cut, whole stalk cane.

Legendre: Yes, you are right but only if there are no other predisposing factors, i.e., killing freeze or intense burn. In the case of this test all stalks were frozen to the ground. On the morning of December 25, all stalks of all varieties were splitting from top to bottom. After thawing, the freeze cracks were not as evident, but the damage had been done.

McCowage: The next point is in the line of dextran analysis. The laboratory results showed that the cane deteriorated quite rapidly throughout the entire period. The haze test showed that dextran increased at quite a different rate than that indicated by the copper test. This may mean there was at the same time a very large increase in other polysaccharides. I was just wondering if you could explain what those polysaccharides were, if they weren't dextran?

Legendre: I am sure that some of the difference in dextran content as reported by the two methods is due to molecular weight of dextran present. The dextran concentration found in L65-69 appeared to stabilize while the concentration of other polysaccharides increased at a more rapid rate; however, I cannot explain what actually occurred.

Stanley E. Bichsel, American Crystal: When you screen your varieties, is there any physiological element that you look for, or biochemical element that gives you an indication that this particular variety might have more resistance to freeze damage than another?

Legendre: No, we have not detected any physiological or biochemical element that we can associate with cold tolerance; however, Dr. Paul Moore, USDA-ARS, Hawaii, has associated drought and cold resistance to free proline content. We do know that following freeze injury, dead and moribund cells become vulnerable to invasion by the bacterium, Leuconostoc mesenteroides. This bacterium consumes sucrose and produces dextran as a by-product. The bacterium's entry into storage tissue is facilitated by dead lateral buds (-4.4°C) and by freeze cracks (-5.6°C). Accordingly, we look for tissue damage as an indication of resistance to freezing temperature but once cane is frozen to the ground as was the case in this study, the only way to detect deterioration is to measure changes in juice quality over time, i.e., dextran, pH, drop in sucrose and purity.

Enrique R. Arias, Sugar Cane Growers Coop. of Florida: To what do you attribute the closeness between the results that you had on apparent sucrose and HPLC sucrose?

Legendre: The closeness of the two readings was somewhat of a surprise. I anticipated that apparent sucrose would have been higher due to the dextro-rotation of dextran. However, that was not the case. In any event, the results of the two methods were quite similar and I am not sure just why.

Arias: I was wondering why the large increase in dextran that you had did not affect the pol.

Legendre: No, there was no apparent effect of dextran on polarization. Apparently, dextran as well as other optically active substances were removed from the filtrate by the clarifying agents, lead subacetate and filter aid.

Clarke, SPRI: There are several reasons that the pol did not go up, and Dr. Legendre has already suggested one. Another reason shows up fairly quickly, and that's the fructose--when you have high concentrations of dextran in the frozen juices you have got lots of fructose as well. Fructose polarizes highly negative--not quite as high as dextran polarizes positive-- but produces a lot of compensation. But there is another reason that's even more important than the fructose for that pol not to have climbed, as we know it does, when we've got lots of dextran. That other reason shows up in the total percentages of polysaccharides, in those very old juices. Some of those were almost solid, and that means they were very hard to clarify in order to take a pol reading. To clarify before making a pol reading, we had to use the Herles lead reagent to remove all that solid material. That meant getting out a lot of polysaccharide and a lot of dextran. It had actually precipitated out, there was so much of it. The remaining soluble material was read as pol. Tremendous amounts of sucrose were lost, as you can see from the data. The reason that the pol did not show so much difference from the HPLC results was twofold, if I may repeat myself. Quite a lot of polysaccharide had precipitated out and wasn't there to be read. The fructose and negative pol material had not precipitated out, and were still all in the sample to be read.

DEXTRAN - AN OVERVIEW OF THE AUSTRALIAN EXPERIENCE

Philip C. Atkins and Robert J. McCowage

CSR Limited

The Australian sugar industry's interest in dextran began in the late 1950's with the advent of mechanical harvesting. As the proportion of the crop which was mechanically harvested grew so too did the effect of cane deterioration on factory processing become more noticeable.

The introduction of mechanical (wholestalk & chopper) harvesting was rapid. In 1961 only 5% of Australian cane was mechanically cut. By the end of the 1960's this had increased to over 80%. By the early 1970's conversion from manual harvesting was practically complete. The successful application of chopper harvesters, with an integrated transport system, was the major reason for this rapid conversion.

In particular mill areas conversion was faster. For example in the 1965 season about 80% of the cane supplied to CSR Limited's two mills in the Herbert River district in Australia was harvested by chopper harvesters.

The severe processing difficulties experienced in Australian factories during this time lead to a concerted research effort which identified dextran as the problem. Research into the control of dextran and the effects of dextran on processing has been continuing in one form or another ever since.

This paper is intended to review the Australian experience so far. Most of the information has been reported before, however we believed it was worthwhile to summarise, for the benefit of people with a current or initial dextran problem, the experiences of the Australian sugar industry over the last 20-25 years.

FACTORS AFFECTING DEXTRAN FORMATION

The formation of dextran in cane was found to be primarily dependent on the following factors.

- . whether the cane is green or burnt
- . the delay between burning and harvesting
- . if chopper harvested, the length of the cane billet, and the quality of the billet cut
- . weather conditions during harvest and transport
- . the delay between harvesting and crushing.

Burning

Deterioration in burnt cane commences at the moment the fire takes place whereas in green cane deterioration commences at harvest.

Foster (1979) concluded that cane was subjected to temperatures as high as 400°C for several seconds during a cane fire and further reported temperatures of 102°C 1 mm below the surface of the cane during a fierce cane fire. He states that the juice in the outer parenchyma and vascular bundles actual boils, perforating the epidermal tissues and thereby leaving the cane stalk open to intrusion and attack by micro-organisms.

Fulcher and Inkerman (1974) found high levels of dextran (up to 5,900 ppm) in some 'sound' burnt cane which had been standing for 4 days. However, no dextran was found in sound green cane.

McNeil and Inkerman (1977) confirmed these findings, and identified a wide range of organisms in long standing burnt cane. *Leuconostoc mesenteroides* was the dominant micro-organism.

In addition to deterioration in standing cane, it has been clearly recognised that post harvest deterioration is much faster for burnt cane than green cane. Foster, Ivin and King (1977) found that the post harvest deterioration in burnt cane could be easily detected after 12 hours whereas there was no detectable deterioration in green cane after 48 hours.

Burn to Harvest Delay

One of the more important effects of burn to harvest delay is its effect on post harvest deterioration. It was found by several workers that the longer the burn to harvest delay, the more rapid is the deterioration after harvest.

Henderson and Kirby (1972) found high dextran levels in some cane less than 16 hours after harvesting when the burn to harvest delay was 36 hours or greater, whereas for cane with a burn to harvest delay of less than 24 hours, similar dextran levels were experienced only after a 20-24 hour post harvest delay.

Keniry and Lee (1967) found that an increase in the delay between burning and harvesting increased the rate of post harvest deterioration. Their results are summarised below:

Burn to Harvest Delay (days)	% Increase in Dextran After Post Harvest delay of 16 Hours
3	50
4	67
5	400
7	450

In other CSR trials at Victoria Mill (Watson 1971) it was found that to contain dextran formation the post harvest delay would need to be reduced by about 2 hours for each 10 hours of burn to harvest delay.

Cane Billet Size and Condition

The deterioration in burnt standing cane was stated earlier to be due to micro-organisms entering the cane stalk following damage caused by the cane fire.

When the cane is harvested, any additional sites created which can allow entry by micro-organisms will increase the rate of deterioration. The number of such sites is determined by the size of the cane billet produced and the extent of bruising or mutilation which occurs. A considerable amount of work has been done on this in Australia.

Keniry, Lee and Davis (1967) reported that the rate of dextran formation in chopped cane was far in excess of that in whole stalk cane. Henderson and Kirby (1971) found that after 20 hours post harvest delay the dextran content in first expressed juice from 7" cane billets was double that from 10" cane billets.

Ivin (1972) measured the deterioration in sound long ($>10''$) billets, sound short ($<10''$) billets and mutilated billets. He found significantly higher dextran levels in the short billets compared with the long and up to three times higher dextran levels in the mutilated billets.

Ivin and Bevan (1973) found that on average the rate of deterioration in short (or mutilated) billets less than 10" long was twice the rate of deterioration in sound billets greater than 10" long. They confirmed these findings by determining the viable leuconostoc counts for each run in their trials. Again they found the fastest rate of bacterial growth for the short mutilated billets.

In trials performed primarily to determine to relative deterioration of green and burnt cane, Foster, Ivin and King (1974) also found an approximate doubling of the rate of deterioration in billets less than 10" long compared with billets greater than 10" long.

Harvesting Conditions

Weather conditions at the time of harvest have a significant impact on the rate of post harvest deterioration.

Keniry, Lee and Davis (1967) showed that a rise in temperature and/or an increase in wet or humid weather caused cane to deteriorate more rapidly. They showed that in cool dry weather significant deterioration did not occur until more than 20 hours after harvest, whereas in hot dry conditions significant deterioration occurred after only 14 hours (Figure 1). In hot and wet conditions, deterioration was even more rapid.

In most seasons in Australia this is observed in practice. In the early part of the season temperatures are cooler and the weather is generally dry. Cane deterioration, even after an extended mill breakdown is not a major problem. However, later in the year, particularly in the northern areas, hot, humid conditions are common and harvesting practices have to be more closely controlled to prevent cane deterioration.

Harvest to Crushing Delay

All work on the effect of harvest to crushing delay on cane deterioration has concluded that cane deterioration and dextran formation increase with time. Keniry, Lee and Davis (1967) found that under all harvesting conditions, dextran formation increases as the post harvest delay increases. The size of the increase and the minimum time that should be allowed between harvest and crushing depends on the combination of all of the previous factors discussed; burning, burn to harvest delay, billet size, billet quality and harvest conditions.

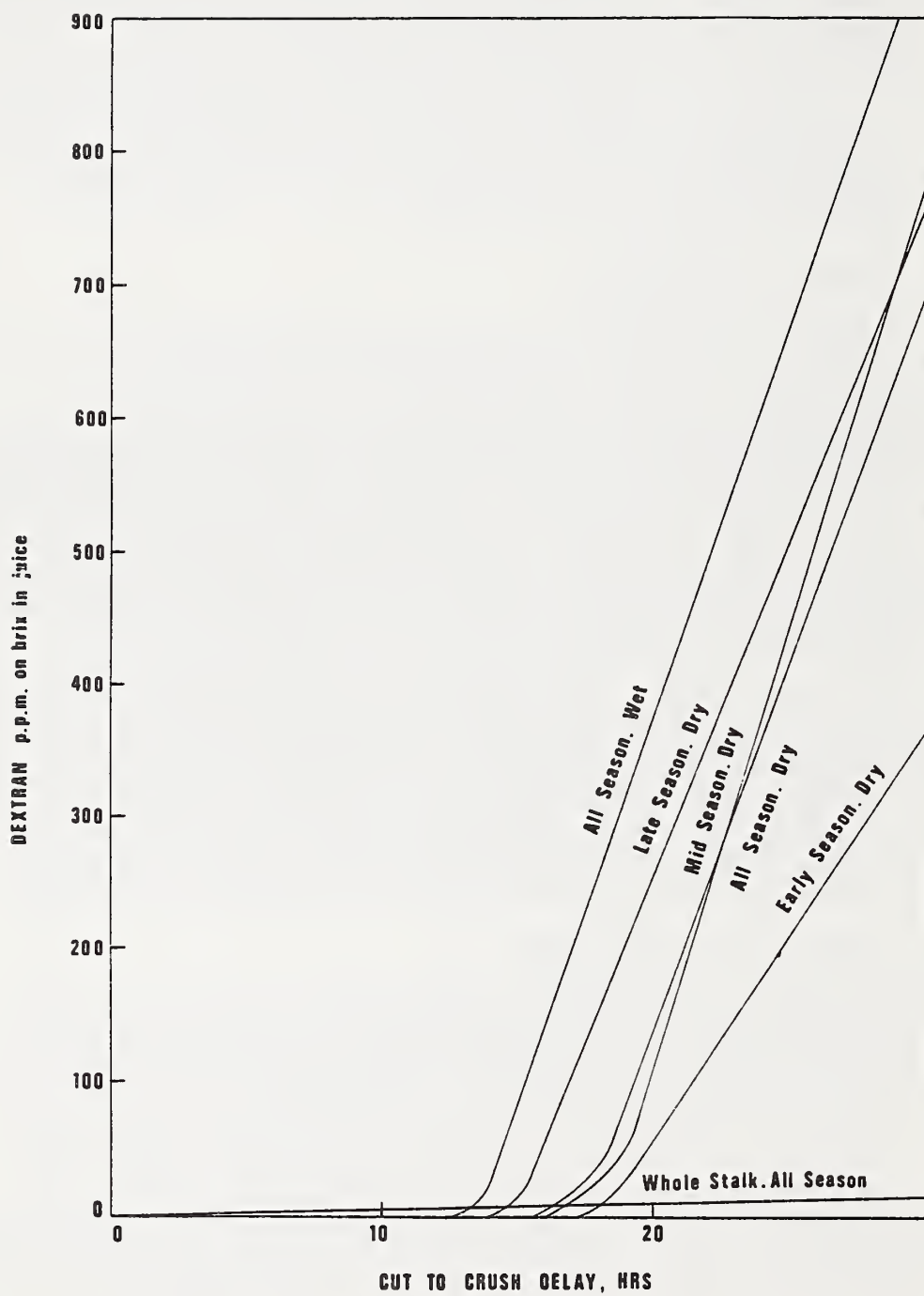


Figure 1.--Effect of weather conditions at harvest on the deterioration rate of chopped burnt cane

Summary of Field Factors Affecting Dextran Formation

A series of graphs by Watson (1971) reporting on work performed at Victoria Mill gives a good summary of the various factors described above. Figure 2 shows a comparison between green and burnt chopped cane under different climatic conditions. Figure 3 shows the relationships between burn to harvest delay and harvest to crushing delay. Figure 4 shows effect of billet length.

We believe the graphs give a fair indication of the relative importance of the various factors affecting cane deterioration and are a fair summary of the Australian experience. Based on this experience, the growing and milling sectors of the Australian industry have co-operated to form a cane harvest and transport operation which minimises the opportunity for cane deterioration. This is based on:

- a) Reduction in Burn to Harvest Delay - daily burns,
penalties for
excessive burning
- b) Reduction in Cut to Crush Delay - integrated
harvest/transport
system to minimise
delay from cut to
crush.

In addition individual mills perform billet quality surveys in which the length and degree of mutilation of cane billets is measured and the information is fed back to the harvester operators.

DEXTRAN IN THE FACTORY

Chemistry and Occurrence

The chemistry of dextrans has been reviewed in the literature (Imrie and Tilbury 1972, Coll Clarke and Roberts 1978). They are polysaccharides containing a majority of $\alpha,1-6$ glucosidic linkages. They can be synthesised by a large number of bacteria (Jeanes 1954) and dextrans derived from different sources vary in the degree of branching, type of branch linkage and molecular weight (MW).

These differences are important as they influence the physical properties of the dextran and hence its effects during sugar processing.

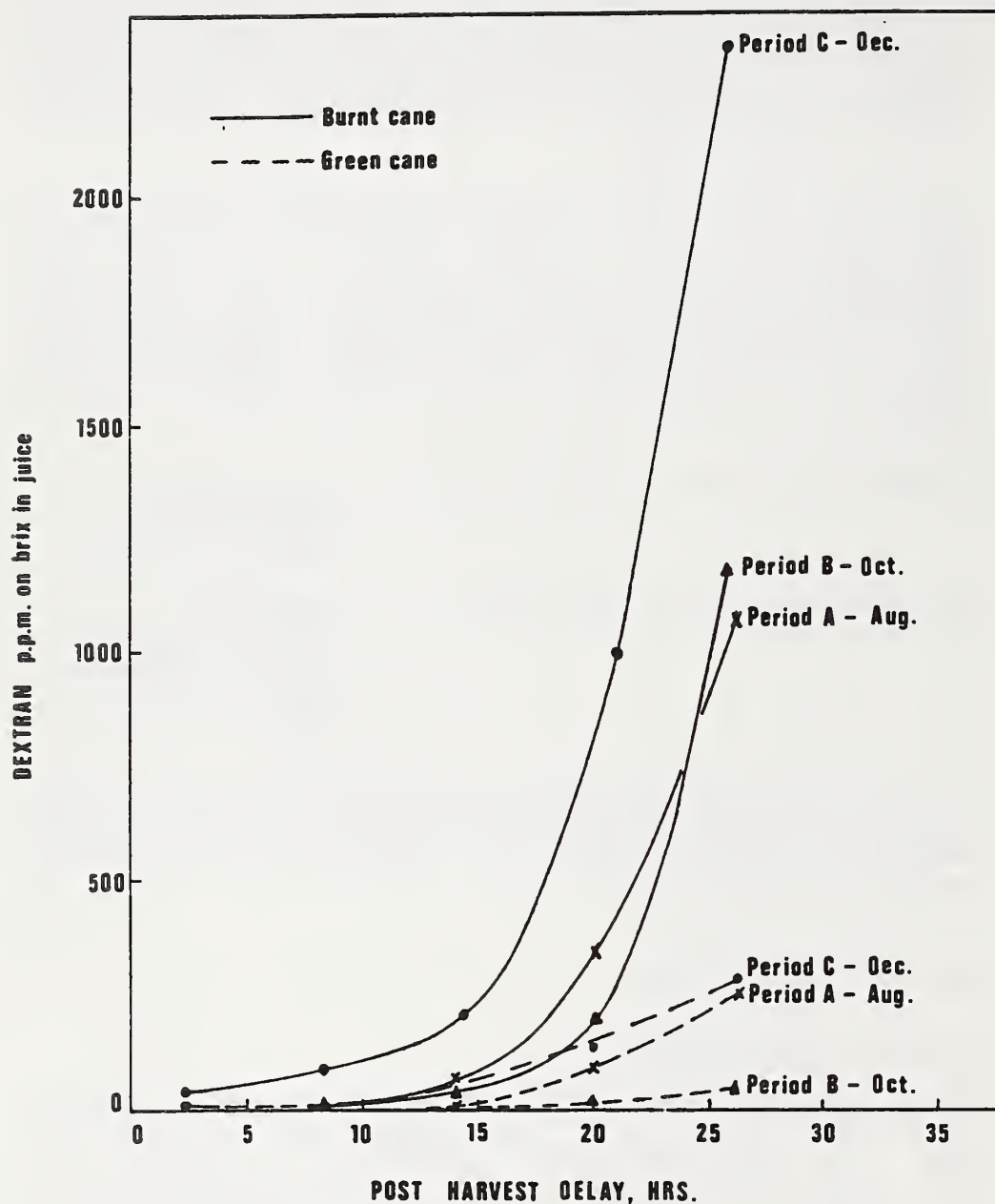


Figure 2.--Effect of burning and climatic conditions on the deterioration rate of chopped cane

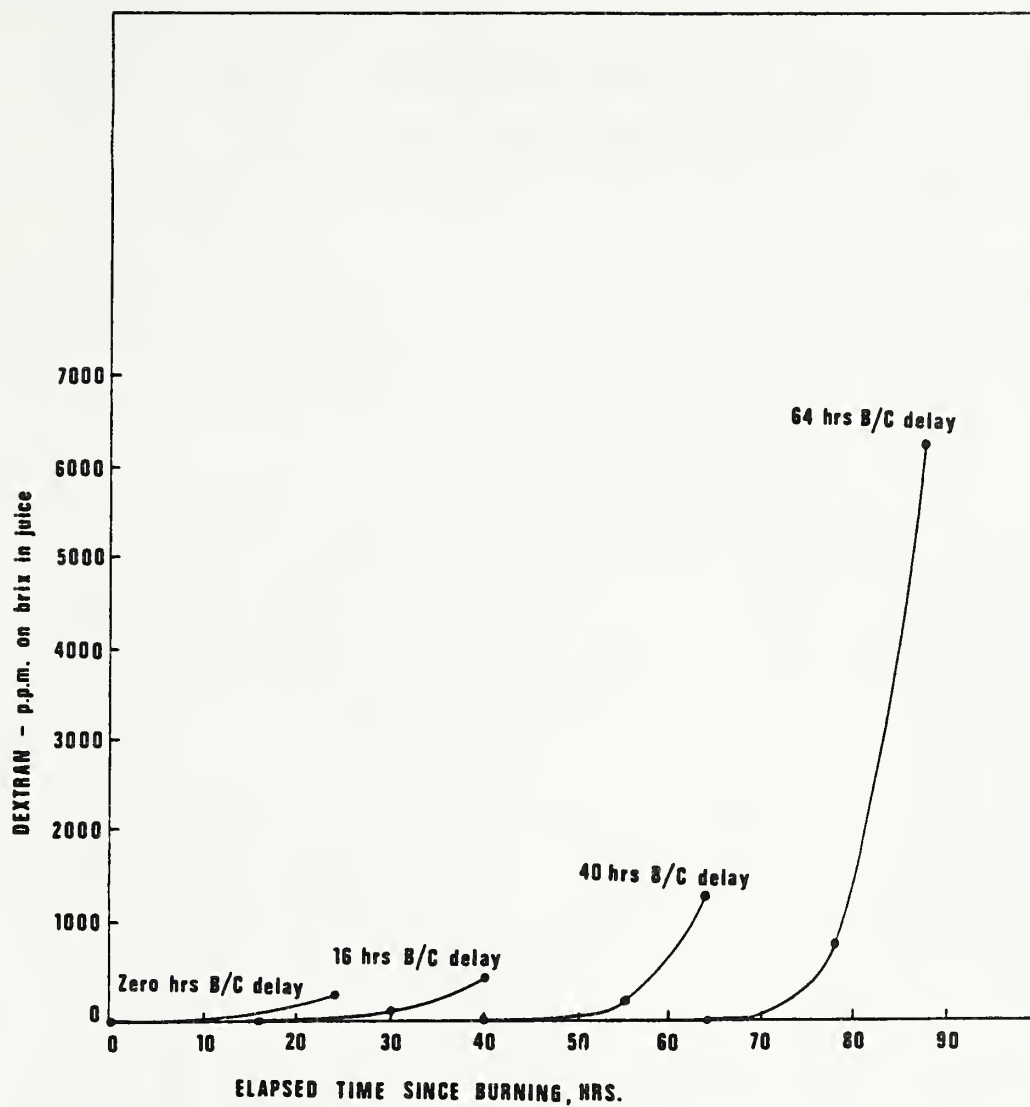


Figure 3.--Effect of burn to cut (B/C) and cut to crush delays on the deterioration rates of chopped cane

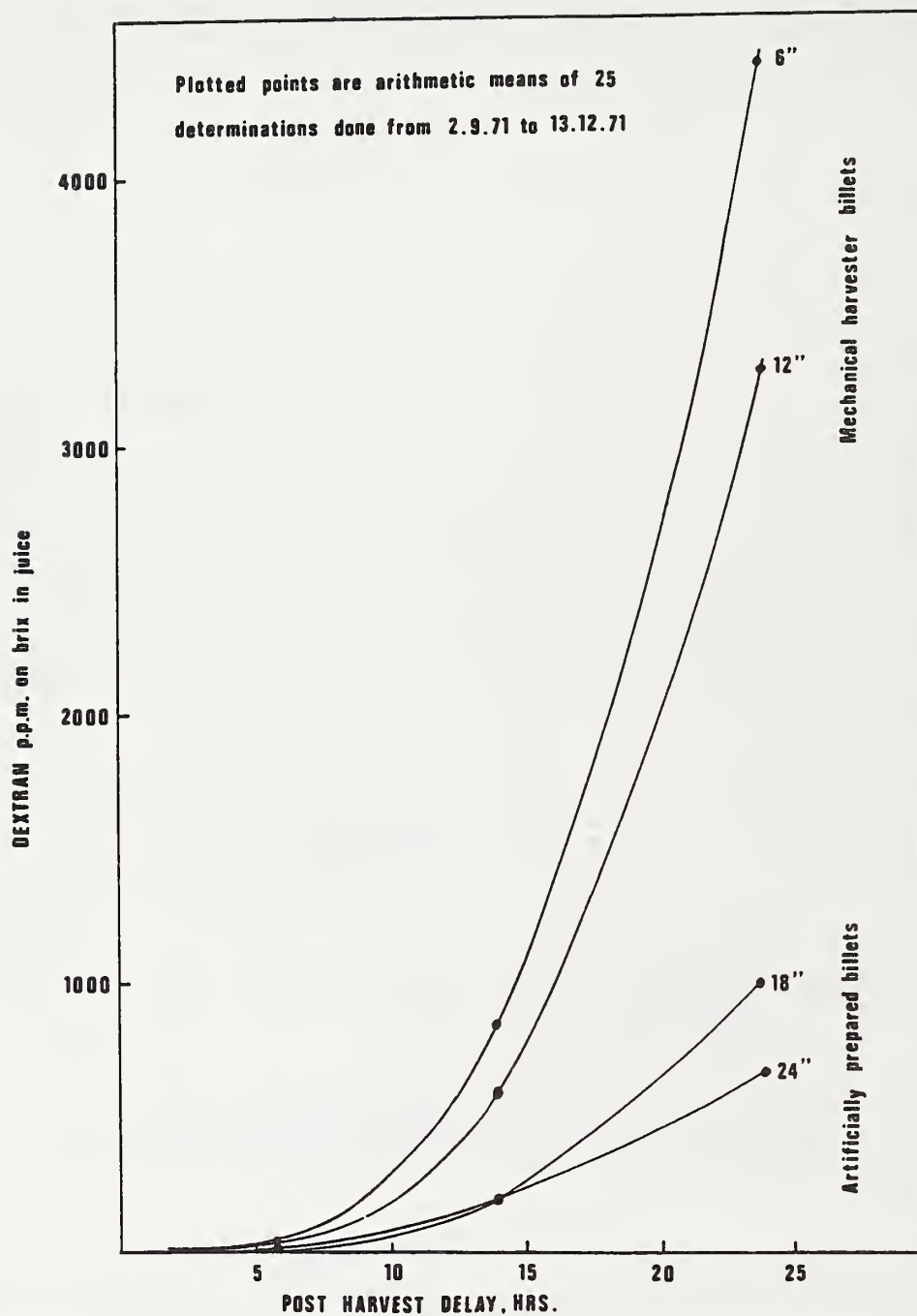


Figure 4.--Effect of billet length on the deterioration rate of burnt cane

Egan (1964) and Forster, Inkerman and McNeil (1977) have shown that *Leuconostoc mesenteroides* is responsible for the dextran produced during cane deterioration in Australia. The average molecular weight is relatively high both in deteriorated cane (Foster et al 1977: 25×10^6) and in sugars produced from deteriorated cane (Covacevich and Richards 1975: 20×10^6) and the structure is fairly uniform with approximately 95% $\alpha,1-6$ linkages and 5% $\alpha,1-3$ linkages, (Foster et al 1977, Leonard and Richards 1975 and Covacevich and Richards 1975).

These findings are consistent with Bovey's (1959) evidence that the reaction in which dextran is formed is end-group catalysed involving a single chain mechanism. The average molecular weight would thus be expected to reach a high value early in the polymerisation process and increase slowly from then on.

Processing Effects of Dextran

A great deal of information has appeared in the literature on the problems experienced by cane sugar millers and refiners during the processing of deteriorated cane and raws produced from deteriorated cane. The Australian experience is not unique in this area although we have never had a refined product dextran complaint as experienced in some countries.

During milling, juice clarification deteriorates as the dextran polymers act as protective colloids and hinder the aggregation and settling of the insoluble particulates. Evaporation and boiling rates drop, elongated crystals appear and the exhaustibility of both high and low grade massecuites deteriorates. The reduction in boiling rate, when coupled with poor exhaustion in the high grade pans and lower purging efficiency of low grade massecuites causes a serious decrease in the effective capacity of pan and centrifugal stations. There is also a greater loss of sugar to final molasses. Chemical control in the factory also suffers due to the effects of dextran on the polarisation determination.

During refining, affination efficiency falls and greater quantities of higher purity recovery streams are produced, filtration rates can fall dramatically. In low grade material processing boiling rates and exhaustibility are affected. Overall chemical control becomes more difficult.

Thus the effects of dextran are many and varied. They are principally associated with the effects of dextran on:

- . viscosity
- . crystallisation
- . clarification processes
- . polarimetry

Each of these have been studied in some detail in Australia.

Viscosity. The effect of dextrans on the viscosity of sugar solutions is arguably the most important of the dextran effects in sugar processing since it directly affects boiling rates, exhaustion and purging.

Laboratory based studies have shown that both the absolute dextran concentration and its average molecular weight are important.

Day (1971) and Greenfield and Geronimos (1982) presented data which clearly demonstrated this fact. Day (1971) found that dextrans with a molecular weight of 40,000 and less had a relatively small effect on solution viscosity even when in concentrations up to 10,000 ppm on solids. His results are reproduced in Figure 5. Greenfield and Geronimos (1982) found that the effect of dextran of 40,000 MW was so small that they disregarded it in developing an expression describing the effect of dextrans on juice viscosity.

$$n_{sp} = 0.036 \gamma^{0.35} C^{1.21} \quad (1)$$

where $n_{sp} = (n - n_{ref})/n_{ref}$

n_{ref} = viscosity of a solution of the same total solids but zero dextran (poise)

γ = weight average degree of polymerisation (MW/162)

C = dextran concentration (wt/vol of solution)

Branching can also play a role as it has been found (Greenfield and Geronimos 1982) that the dependence of viscosity on the molecular weight of a particular system is weaker the higher the degree of branching.

Crystallisation. A number of studies were undertaken to assess the effect of dextran on crystal elongation and growth rates. Again concentration and molecular weight were found to be important.

Keniry, Lee and Davis (1967) found significant correlations between dextran concentration and crystal elongation. This was taken further by Sutherland and Paton (1969) who confirmed that dextran promoted c-axis elongation but also that the extent of the elongation was enhanced by raising the temperature of the solution and the molecular weight of the material. Leonard and Richards (1969) confirmed that oligosaccharides are not responsible for the severe elongation found when processing deteriorated cane.

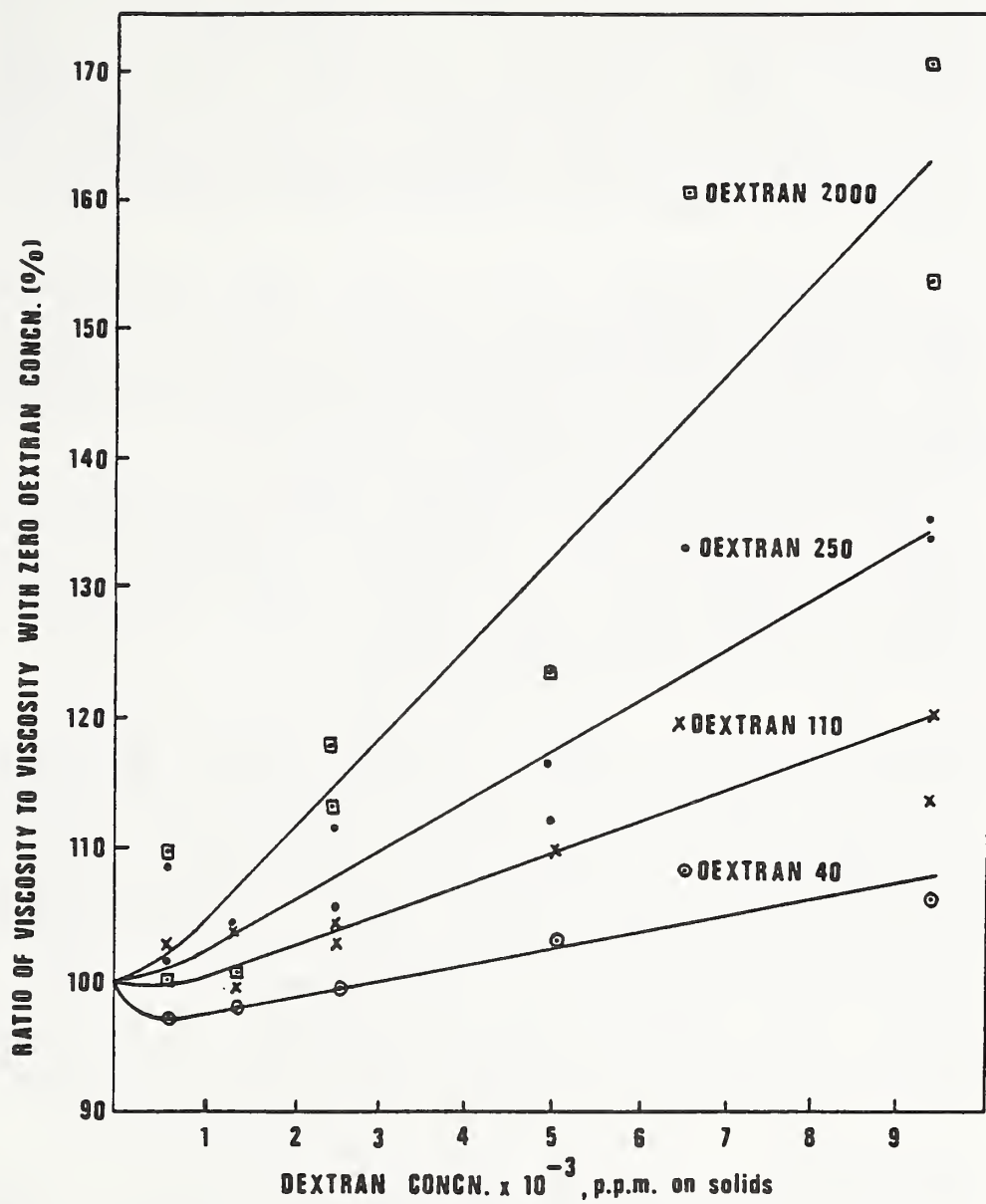


Figure 5.--Effect of dextrans on the viscosity of sucrose solutions

Day (1971) confirmed the results of earlier investigations concluding that dextran of a molecular weight of 40,000 could cause elongation but not to the same extent as higher molecular weight materials. He also reported similar findings concerning crystal growth rates. At a concentration of 1% by weight of solids, dextran of 2 million MW reduced growth rates by 35% while 40,000 MW dextran reduced the rate by only 18%. Results are presented graphically in Figures 6 and 7.

Clarification Processes. While Davis (1959) suggested that dextran acts as a protective colloid and inhibits coagulation thus allowing fine suspended matter to pass out of the clarifiers with clarified juice no detailed studies were even undertaken to attempt to quantify these effects.

On the other hand Hidi and McCowage (1984) have reported the results of studies on the effect of dextran on filtration rates in carbonatation refineries. Concentration and molecular weight again are important with the effects of dextran on filtration rates only becoming important at molecular weights approaching 4 million.

This work did not address the matter of the effects of dextrans on direct filtration however Fulcher and Inkerman (1978) reported work which it is believed would be relevant. He added commercially available dextrans of different molecular weights to refined sugar solutions and determined the effect on the filtrability of the solution using the Nicholson and Horsely (1956) test, a direct pressure filtration test using a diatomaceous earth filter aid. It was found that dextrans up to 2 million MW had no effect. In contrast B512 dextran and native dextran had significant effects. When these dextrans were added to raw sugar solutions the scale of the effects was considerably reduced.

Polarisation. The influence of polysaccharides on polarisation has received considerable attention in world literature. It can be concluded that with dry lead clarification the polarisation of juices and mill process streams will be increased by the presence of dextran. The extent of the increase will be proportional to the amount of dextran present but less than that predicted by theoretical considerations based on the specific rotation of pure dextran (Guzman 1977, Tantaoui 1962, and Mellowes 1979). This is not surprising as the precipitate which is formed in situ adsorbs and entraps large molecules present.

Thus polarisations determined in the usual fashion will be directly affected by the presence by dextran. Juices up to 1000 ppm dextran can clarify normally (Wells and James 1976)

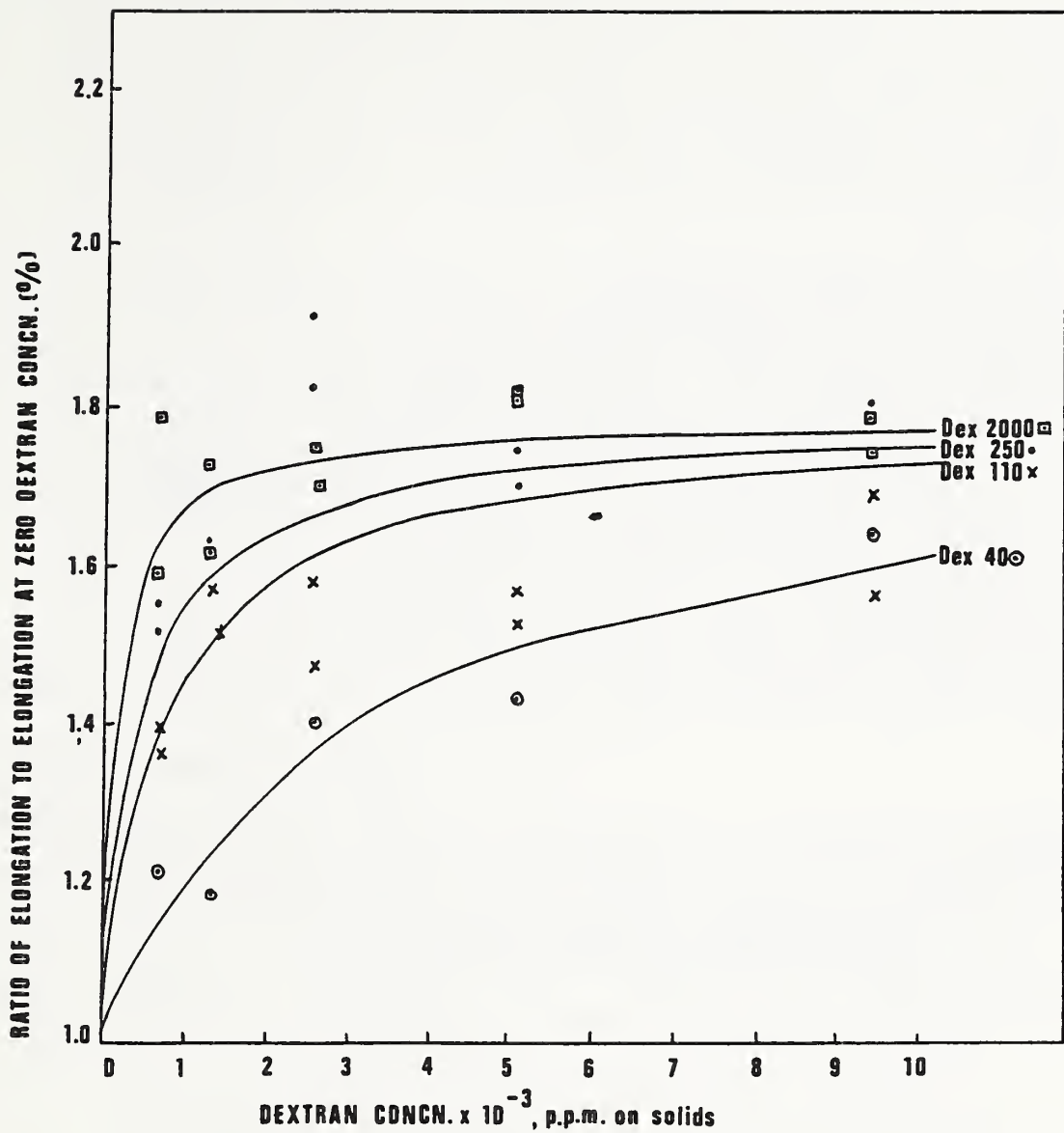


Figure 6.--Effect of dextrans on crystal elongation

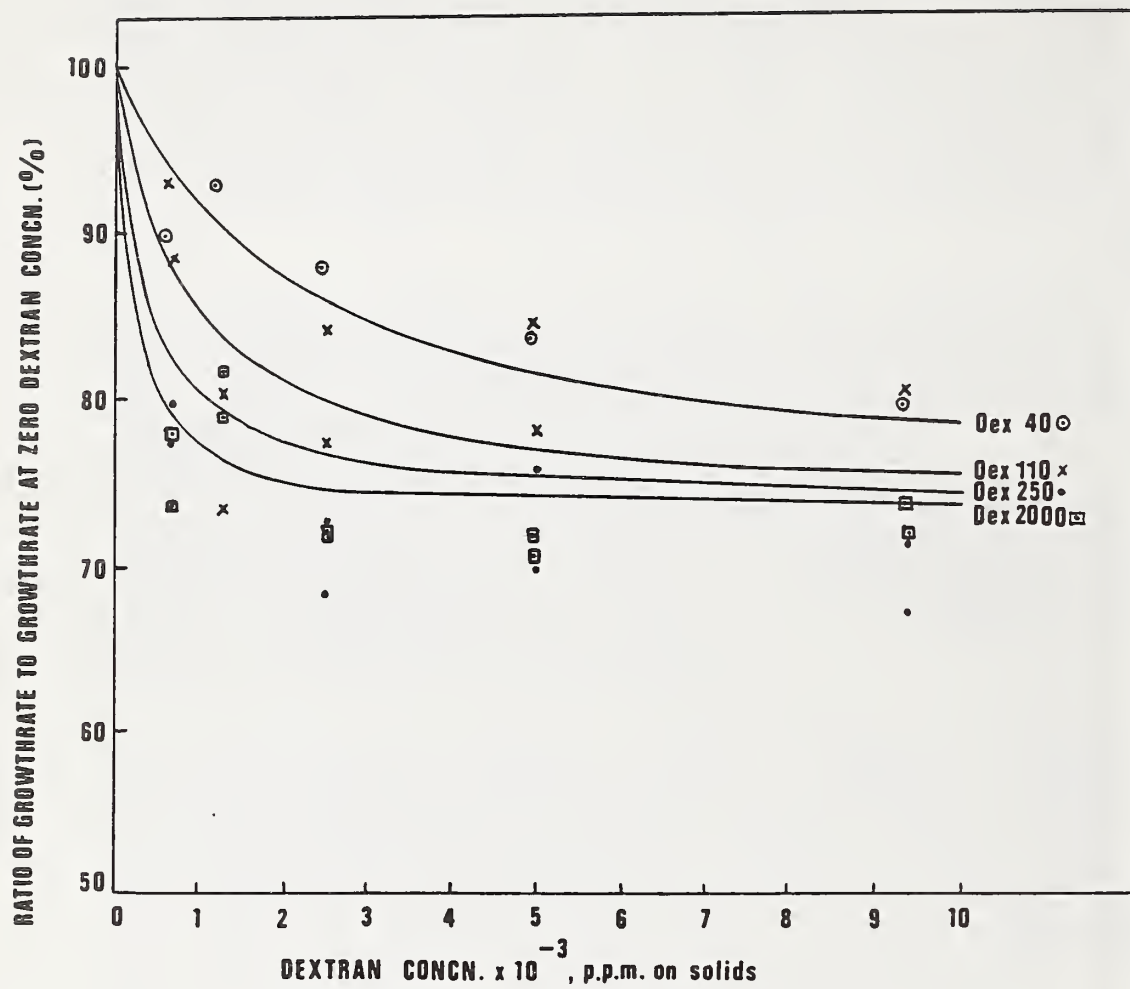


Figure 7.--Effect of dextrans on crystal growth rates

and so this effect can be unnoticed at low to moderate dextran levels. As dextran levels rise further sample clarification becomes more difficult and often more lead is required to obtain the clarity required for the polarisation determination. This additional lead can of course cause its own problems due to its removal of fructose from solution (Mellowes 1979). Herles reagent can be successfully applied to clarify these juices and this also eliminates interference from dextran.

The effect of dextran on raw sugar polarisation has been investigated in Australia (CSR unpublished). Polarisation determinations were carried out on five raw sugars after the addition of the standard dextrans T-20, T-110 and T-2000 at 100, 500, 1000, 1500 and 2000 ppm. The averaged results for each of the standard dextrans are presented in Figure 8. With the exception of the lowest dextran concentration used polarisations of the raw sugar solutions increased as the concentration of added standard dextran was increased and this increase was independent of the molecular weight of the dextran added. However, the increase was not consistent with theoretical predictions of three times the percent dextran concentration (Chou and Wnukowski 1980) as some dextran is removed during the standard lead subacetate clarification procedure just as it is with similar treatment of juices.

Processing Options

Once dextran is in the factory, be it sugar mill or refinery, it is our experience that conventional processing offers little opportunity to relieve the situation. The best one can do is minimise the further formation of dextran in process, aim for the best clarification possible under the prevailing conditions, and attempt to minimise recycle streams.

In minimising the further formation of dextran in process particular attention needs to be paid to sanitation and minimisation of intermediate process stocks particularly low brix solutions at temperatures below 60°C.

Fortunately dextran related problems can be relieved in the sugar mill as dextranase can be used to good effect in the treatment of deteriorated cane. Inkerman (1980) reviewed the Australian industry experience with dextranase. Application has been confined to sugar mills. It is not used in Australian refineries. Its application in milling is as a last resort only.

Juice is incubated with the enzyme at its natural pH at 57°C for approximately 40 minutes. When applied to a factory processing deteriorated cane and completely removing

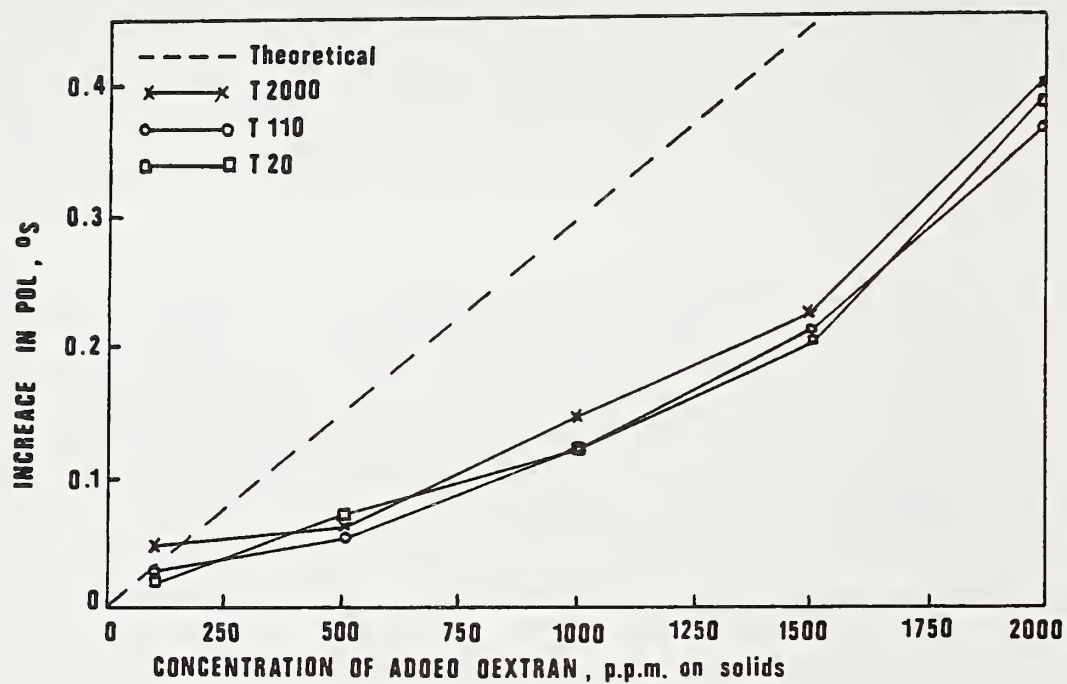


Figure 8.--Effect of dextran on raw sugar polarisation

dextran, clarified juice turbidities improve dramatically, crystal elongation is minimal and viscosities and final massecuite boiling rates return to normal (Fulcher and Inkerman 1977).

With partial dextran hydrolysis, viscosities return to normal however grain elongation will still be present. Importantly however, normal factory throughput can be achieved.

These results are consistent with results reported earlier which show that the viscosity effect of dextran drops rapidly with molecular weight while the crystal elongation effect, although diminished, can still be significant with dextrans of 40,000 MW.

The action of dextranase can be appreciated when one considers work carried out by Hidi (CSR unpublished), who treated a high dextran mill syrup with dextranase and monitored the change in concentration of different dextran fractions with the progress of the enzymed action. Results are presented in Figure 9, and show the rapid breakdown of the high molecular weight material and formation and then gradual degradation of intermediate fractions. Given sufficient time all fractions would be eliminated.

DEXTRAN MEASUREMENT

As already discussed dextran became a major problem for the Australian industry during the nineteen sixties with the change to mechanical harvesting. Considerable field losses and factory processing difficulties were encountered but it was not until dextran was found to be a quantitative indicator of the problem (Keniry, Lee and Davis 1967) that it became possible to understand and develop the means to control it.

The CSR Haze Test

The original haze test was developed by Nicholson and Horsley (1959). It was adapted by Keniry, Lee and Davis (1967) to ensure uniformity between laboratories in the standardisation procedure and to overcome interference from inorganic salts. It was further revised in 1969, (Keniry, Lee and Mahoney 1969), with changes being made to the incubation and ion-exchange steps, the latter with the aim of overcoming batch differences in resins as supplied, and excess removal of cations over anions. The haze test has been successfully used in that form by the Australian industry to provide the basis for controlling dextran for some 14 years.

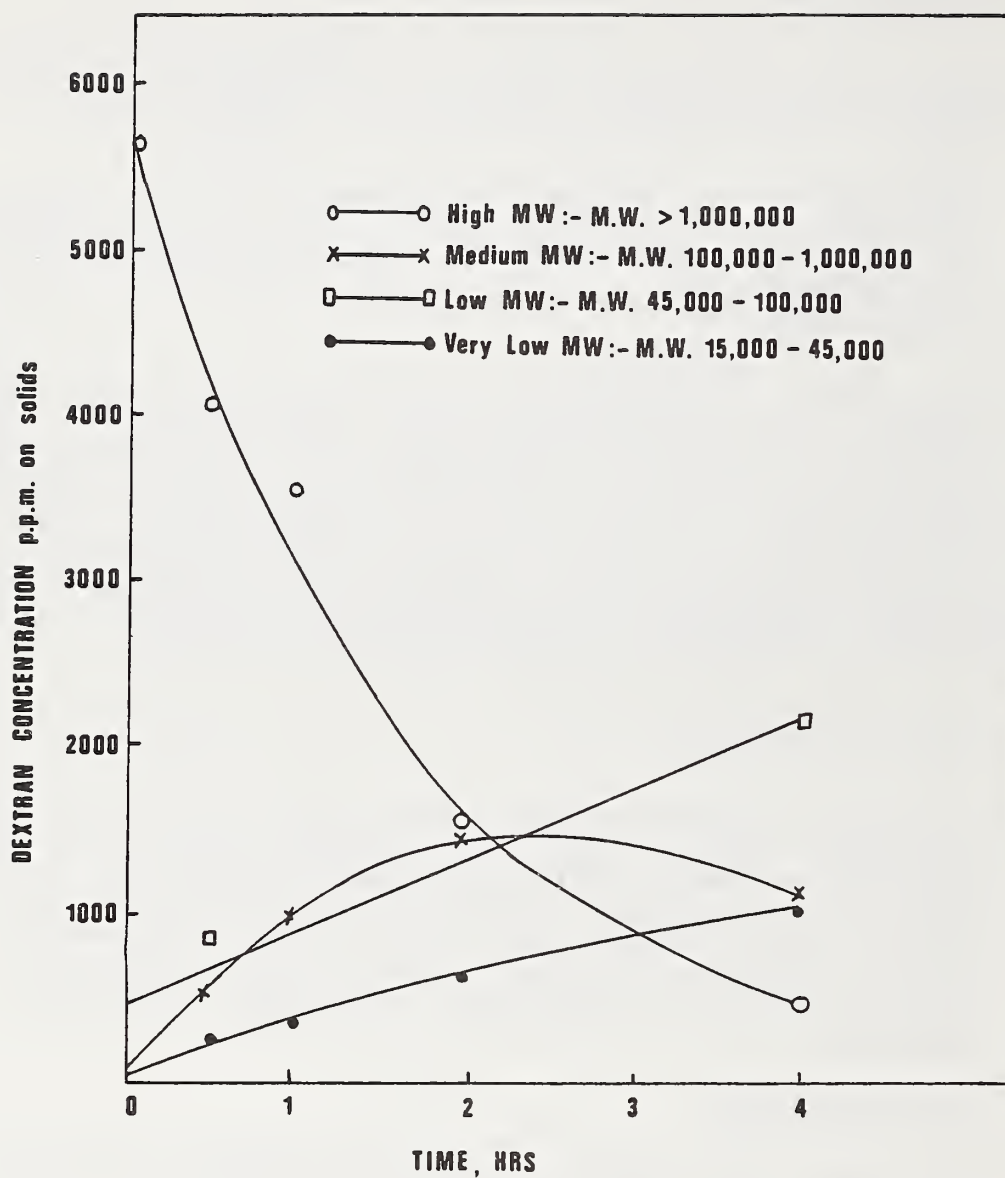


Figure 9.--Effect of dextranase treatment on the concentrations of different MW dextran fractions

During the past 10 years the haze test has been widely criticised in the literature. The main causes of discontent have been lack of sensitivity to low molecular weight dextrans and lack of specificity for dextran itself. A number of alternate procedures have been put forward.

It is conceded that the haze test would not be acceptable for a scientist who wished to study pure compounds with high precision, however, the above criticisms do not hold up in practice because the test does give a reliable estimate of the high molecular weight dextran fractions which have been demonstrated to be responsible for the major processing difficulties associated with this polysaccharide.

The variation of haze characteristics of dextrans of different molecular weights was fully appreciated by Keniry, Lee and Mahoney (1969) when they published improvements in the dextran assay procedure. Figure 10, reproduced from that paper, clearly demonstrates this point.

This can also be seen in tests undertaken in at CSR laboratories to investigate the effect of dextran molecular weight on haze formation in different ethanol water mixtures. The relative insensitivity of haze formation to lower molecular weight dextran is readily apparent in the results presented in Figure 11.

The optical behaviour of the haze formed at different alcohol concentrations is different. Each would need its own calibration curve. However there is no significant difference in the calibration of optical density versus dextran concentration in the same alcohol water mixture using dextrans of different MW if the dextran MW is above the minimum value characteristic for the precipitation in the alcohol mixture concerned. In 50% alcohol this minimum value is approximately 100,000 dalton MW.

Thus this claimed limitation of the haze test has always been recognised. It has not been of practical concern because of the points raised earlier concerning the molecular weight of native dextrans isolated from deteriorated cane and sugars produced from deteriorated cane in Australia and the importance of molecular weight to the processing characteristics of high dextran process streams.

The other major concern expressed about the haze test has been in relation to its lack of specificity for dextran. This also is accepted as other polysaccharides can be precipitated in a 50% ethanol solution. However, once again in a practical context this concern has been shown to be unfounded in Australia. Hidi, et al (1976) showed that the

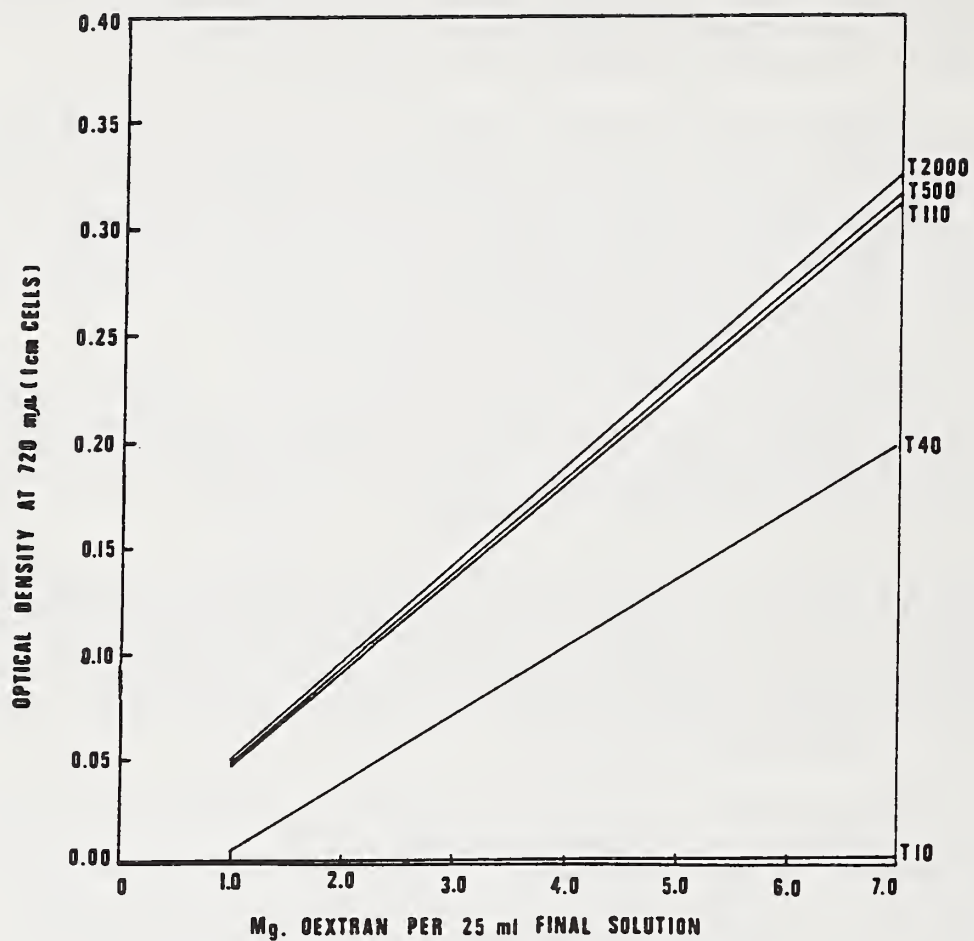


Figure 10.--Standard curves for different dextrans - method of Nicholson and Horsley

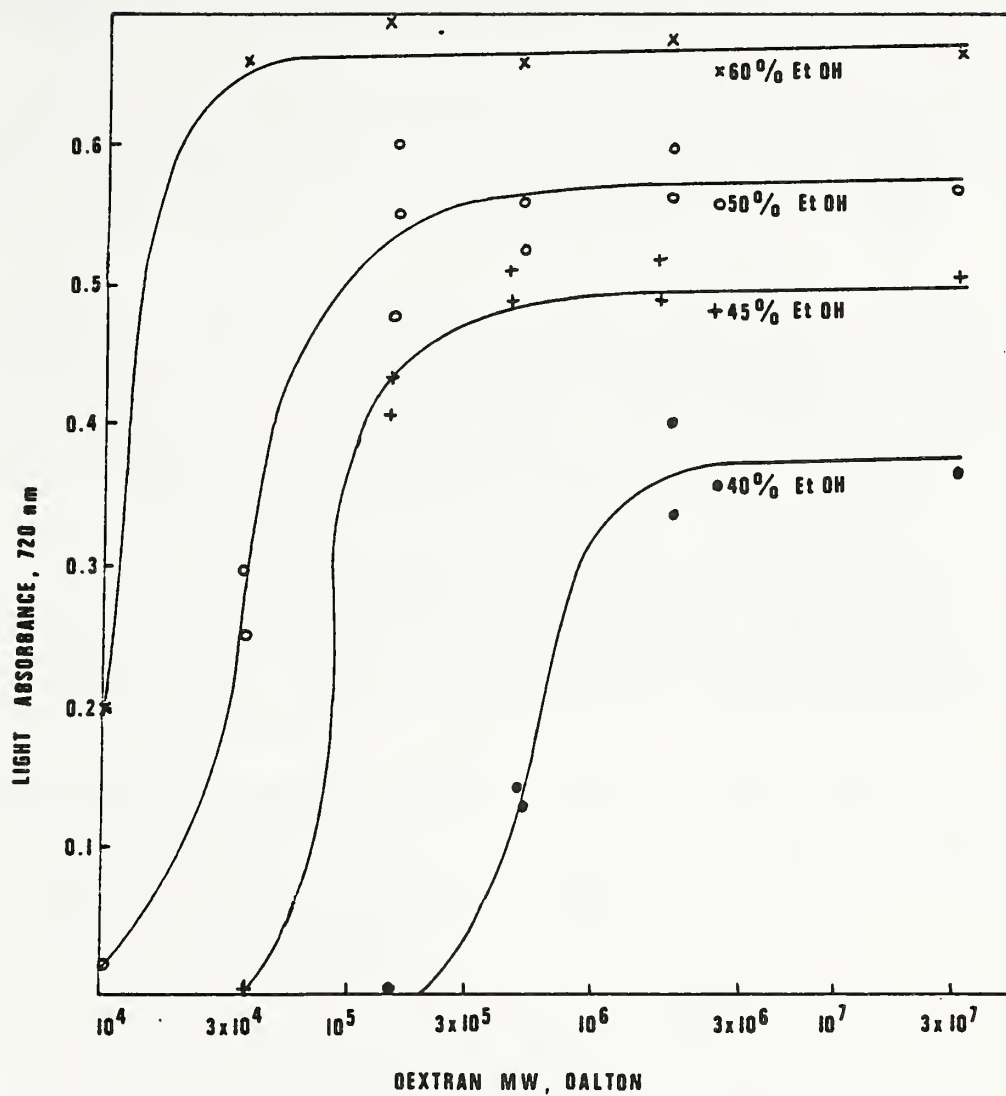


Figure 11.--Effect of dextran molecular weight on the haze formation in different ethanol water mixtures

haze produced by this test was predominantly an α -1,6 linked glucose polymer. Furthermore wide ranging application of the test at 11 mills in Fiji and Australia over a period of 5 years found that a positive result by the haze test was rarely, if ever, observed for juice from freshly harvested burnt or green sound cane.

Also enzymic confirmation that the haze is essentially all dextran was reported in deteriorated cane juice studies (Fulcher and Inkerman 1974) and in raw sugar filtrability studies (Fulcher and Inkerman 1978). Data for the latter trials are reproduced in Table 1.

While these results may be surprising to some, it should be borne in mind that not all polysaccharides will necessarily produce a significant interference with the haze result. Starch is of course removed prior to haze development but hemicellulose is an inefficient haze producer, pectin produces no haze, and galactomannans tend to form precipitates. (Covacevich, Richards and Stokie 1977).

Of course it is not suggested that the haze test could not be improved upon. It does lack sensitivity in the 0 to 100 ppm dextran range and even Hidi et al (1976), despite their assertions about the relevance of the haze test, found in all samples analysed some galactose, mannose and other sugars besides glucose after hydrolysing the dextran haze.

Table 1.--Effect of enzymic removal of dextran on haze result

Sample	Dextran (ppm on Brix)	
	Untreated	Treated
1	960	60
2	1220	50
3	1140	NIL
4	820	NIL
5	490	60
6	875	NIL
7	520	NIL
8	975	50
9	1065	50
10	730	50
11	820	110
12	1025	50
13	875	NIL
14	820	50

Also, dextrans of molecular weights less than those quantitatively measured by the haze procedure are not without effect on crystal elongation and polarisation. While significant concentrations of dextrans of this molecular weight would not normally be present in material derived from deteriorated cane, if dextranase was used to partially treat this problem in the sugar mill then relatively high levels of these fractions could be present.

Alternate Procedures

Increased awareness of the dextran issue has led to the development of a number of alternative procedures for dextran measurement. These include an enzyme dialysis procedure (Richards and Stokie 1974), immunochemical procedures (Hellsing, Enstroim and Richter 1976, Goodacre and Martin 1981), enzyme haze procedures (Ivan and Bevan 1973, Fong and Mbaga 1982), an enzyme total polysaccharide procedure, (Atlantic Sugars Limited, unpublished), an enzyme viscosity technique for juice analysis (Greenfield and Geronomos 1978), an HPLC approach (Wong-Chong and Martin 1980), a modified haze procedure (Chou and Wrukowski 1980) and a copper sulphate precipitation method (Roberts 1983).

Different results invariably arise using the different analytical procedures. This is caused by variability in the composition of dextrans present in the sugars, differing sensitivities of the various methods to the molecular weight ranges of the dextrans and differing procedures used to estimate and standardise the particular analysis.

In considering the applicability of particular methods it is important to consider the reason for which the measurement is being undertaken. It is suggested that this is to give an indication to factory personnel of the processing quality of the material in question, whether it be cane juice, syrup or raw sugar. As such the method should be simple, robust and sensitive to the dextran fractions that will cause processing difficulties.

If one considers the basis of the methods that have been proposed for dextran analysis in the light of these comments then certain observations can be made :

- (i) haze methods standardised against low molecular weight dextrans overestimate the levels of high molecular weight fractions. These would normally be expected to represent the bulk of the dextran in juices or raw sugars and are mostly responsible for the processing problems associated with them.

- (ii) procedures which include an enzyme treatment offer the possibility of improved specificity as long as a pure enzyme is used. Total specificity cannot be assured however as there are polysaccharides other than dextran which originate from sugar cane and which contain $\alpha,1-6$ glucosidic linkages.
- (iii) immunological procedures offer perhaps the best opportunity for specificity. Suitable instrumentation is readily available at a moderate price and it is feasible to produce the necessary antigen in sufficient quantity. However, work published to date indicates that low molecular weight dextrans exhibit a larger response than high molecular weight dextrans. Also dextran from different sources would give further variations.
- (iv) any procedure which is independent of the molecular weight of dextran will give equal weighting to low and high molecular weight material. The effects of different dextran fractions are clearly not the same and some measure of the molecular weight range of the dextran molecules present is obviously needed if a realistic interpretation of results is to be made.
- (v) any procedure which uses a phenol-sulphuric acid reaction for quantification is likely to be difficult to employ in a factory or routine situation. The phenol-sulphuric acid procedure is not held in high regard amongst analytical chemists mainly because of its extreme sensitivity to any organic contaminant, including dust and the need to follow all procedural steps exactly, including the rate of addition of reagents and sizes of glassware, if reproducible results are to be obtained.
- (vi) procedures using copper sulphate to precipitate dextran from a mixture of polysaccharides need not be specific. Fehlings solution complexing has been used for many years to purify different carbohydrate mixtures. Specificity will depend on the structures and concentrations of all the polysaccharide species present.

A number of these points can be demonstrated by considering the results obtained from the analysis of sugars by different procedures.

Workers in CSR analysed a selection of sugars by the CSR haze test, the Fong and Mbaga enzyme haze procedure standardised with Pharmacia T40 dextran and the enzyme total polysaccharide test as undertaken by Atlantic Sugars Limited. Results are presented in Table 2.

Both enzyme procedures tend to give higher results than the CSR haze test. The enzyme haze results are elevated because of sensitivity to a wider range of dextran fractions, the use of an impure dextranase (the Novo 25L specified has some amylase activity - Inkerman private communication) and, most importantly, because of the overestimation of high molecular weight material because of the use of the 40,000 molecular weight standard. The enzyme total polysaccharide results are elevated because of the same sensitivity to a wide range of dextran fractions and lack of specificity of the enzyme.

Comparing the results of this procedure to those of the other two tests shows some unexpected relativities. This is believed to be due to difficulties associated with carrying out two accurate measurements of total carbohydrate using the phenol-sulphuric acid procedure before calculating the dextran level by difference. This assumes special significance at low dextran concentrations.

Table 2.--Comparison of dextran analysis procedures

Sample	Dextran, ppm		
	CSR Haze	Enzyme Haze	Enzyme Total Polysaccharide
1	251	280	280
2	395	360	360
3	10	150	60
4	10	80	200
5	10	190	140
6	64	170	660
7	290	320	200
8	112	250	40
9	583	900	260
10	176	170	60
11	10	40	140
12	14	140	240
13	172	150	860

Roberts (1983) presented a comparison of results by the CSR haze test and the copper sulphate method. The copper sulphate test results were higher than the haze test results. This was expected by Roberts as "the copper method determines total dextran present".

CSR workers also have compared the copper sulphate and haze methods. The average results from duplicate analyses are presented in Figure 12. While there may be some tendency for higher levels to be obtained with the copper test when the haze test results are low, the scatter is so great that drawing general conclusions is difficult. Considerable difficulty was experienced in obtaining reproducible results with the copper sulphate method. The standard deviation of the copper sulphate procedure obtained when analysing these sugars was 81 ppm. The standard deviation of haze test results was 7 ppm. No doubt method familiarity could explain some of this difference but certainly not all of it.

Small quantities of the copper-polysaccharide precipitate were isolated from two sugars using this procedure. These were hydrolysed and analysed by GLC after the formation of aldonitrile acetate derivatives. Results are presented in Table 3. As expected the precipitate was mainly glucan however other sugars were present in concentrations not unlike those that are obtained by similar analysis of the haze developed by the CSR test (Table 4 - Hidi et al 1975).

Table 3.--Analysis of copper-polysaccharide complex from copper test

Sugar Component	Sample 1 % by Weight	Sample 2 % by Weight
Arabinose	2.6	0.7
Xylose	0.6	0.3
Ribose	0.5	0.2
Mannose	3.0	2.6
Glucose	92.4	95.1
Galactose	1.2	1.1

CONCLUSION

It seems that a number of raw sugar producers experiencing the same problems as the Australian industry did in the nineteen sixties - increasing labour costs making the introduction of mechanical harvesting an economic necessity and this, with established cane harvest and transport systems, leading to high levels of dextran formation and the associated sugar losses and processing difficulties.

Dextran has been largely under control in Australia for many years. Of course we can hit some rough patches. Mill breakdowns, wet weather, derailments and the cost of

Table 4.--Sugars in hydrolysate of dextran haze from 14 mill process materials

Sample			Sugars, % by Weight			
No.	Type	Dextran Content	Glucose	Galactose	Mannose	Other
1	A Mass	90 ppm	93,5	2,6	3,4	0,5
2	A Mass	70 ppm	92,5	2,5	3,9	1,1
3	A Mass	30 ppm	92,0	3,0	4,0	1,0
4	A Mass	120 ppm	92,6	3,1	3,8	0,5
5	Syrup	1,830 ppm	98,5	0,3	1,0	0,2
6	Syrup	580 ppm	67,5	9,2	11,2	12,1
7	Syrup	320 ppm	86,1	4,2	6,6	3,1
8	Syrup	320 ppm	91,2	4,7	3,8	0,3
9	Syrup	350 ppm	95,0	2,2	2,6	0,2
10	Syrup	410 ppm	88,6	5,6	4,9	0,9
11	Syrup	450 ppm	76,9	10,8	11,2	1,1
12	A Mass	3,400 ppm	95,8	1,6	2,4	0,2
13	A Mass	2,310 ppm	92,4	2,1	4,5	1,0
14	A Mass	2,530 ppm	99,1	0,1	0,7	0,2

dextranase addition mean that at times we have to process high dextran juices. We direct a lot of this sugar to domestic refineries. They don't like it but they don't get surprises. They know what to expect from the analysis of the sugar they are to receive. At times we are even forced to export sugars of a higher dextran content than we would otherwise choose. However, we believe that with over 20 years experience with dextran we have the appropriate means to select the best available sugar to fulfil these obligations. We firmly believe that the practices and procedures developed in Australia could be successfully employed elsewhere to control this problem.

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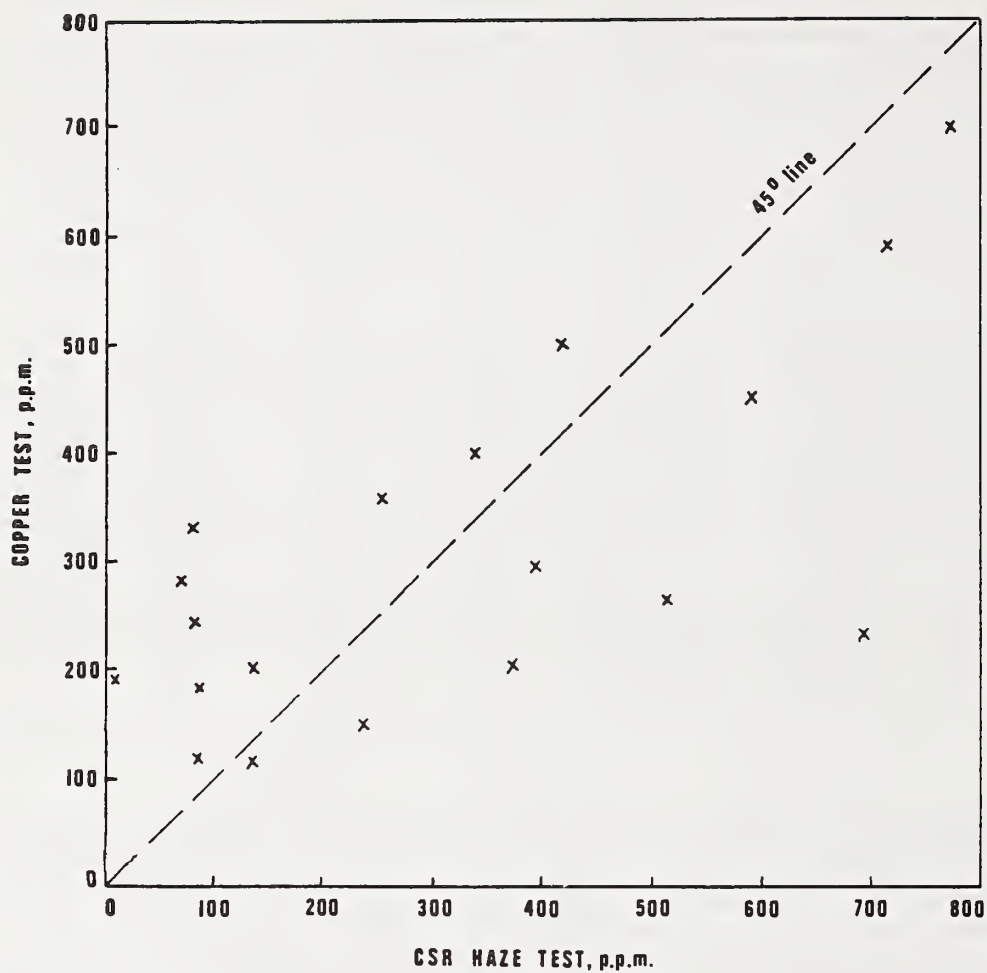


Figure 12.--Comparison of CSR haze and copper sulphate test dextran results

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LEUCONOSTOC SPP. IN SUGARCANE PROCESSING SAMPLES

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INTRODUCTION

Surveys of bacterial populations in cane juice (Meade, 1977; Moroz, 1963; Skole, 1976) have identified three general types: (1) slime-producing species, including Leuconostoc, (2) aerobic spore-formers, and (3) aerobic non-spore formers. Aerobic spore-formers such as Bacillus spp. can predominate in fresh cane juice (Moroz, 1963). However, the Leuconostoc spp. routinely become a major presence during the handling of processing material, as the juice enters the factory. Aerobic non-spore formers in juice are predominantly species of Micrococcus, Flavobacterium, Achromobacterium and Escherichia.

Leuconostoc spp. are responsible for the production of dextrans from sucrose (Egan, 1966). Problems created in processing by dextrans including loss of sucrose are well-documented and represent a major economic problem. (Keniry, 1967; Coll, 1978; Atkins, 1984). In addition, dextrans increase viscosity of juices and syrups that block lines, pumps and filters, lower heat exchange, slow down evaporation, decrease efficiency and yield of crystallization, distort crystal shapes, block centrifuges, and raise loss of sucrose to molasses. Dextrans also increase polarization readings and can interfere with sucrose analyses.

Leuconostoc can infect cane in the field before harvest if there is any wound to the stalk (as from burning, insect damage or freeze damage), and after harvest through the exposed stalk. The longer the delay time between burning and cutting, and between cutting and crushing at the mill, the greater the time opportunity for growth of Leuconostoc and associated levels of dextran that will enter the factory. Ambient temperatures above 25° C and rainy weather encourage growth of Leuconostoc and levels of dextran in post-

harvest cane.

Leuconostoc spp. enter the factory in the cane and reproduce in cane juice during milling and juice collection. Leuconostoc can infect juice at any point in processing and continue to reproduce when conditions are appropriate. However, there has been some question as to whether infection and/or growth will occur after liming.

LEUCONOSTOC SPECIES

Species in the genus Leuconostoc are asporogenous, gram positives in the family of Streptococci. Cells may be spherical but are often lenticular, with frequent pair and chain formation. Routinely, cells are non-motile and non-pigmented, forming small colonies (usually less than 1 mm diameter) that are grayish-white in color. The group is characterized by being facultatively anaerobic with acid production, primarily of lactic and acetic acid, from fermentable carbohydrates. Cells of the genus can grow between pH 5.5 and 6.5, but rarely below pH 5.0. Two closely related species, L. dextranicum and L. mesenteroides are capable of dextran production from sucrose but L. mesenteroides usually is considered a more prolific producer of the carbohydrate polymer. Both species ferment xylose but L. dextranicum generally does not metabolize arabinose; tests for species determination have been developed based on utilization of arabinose (Buchanan and Giffons, 1974). Both species exhibit complex nutritional requirements but L. mesenteroides is somewhat less restricted in substrate needs than L. dextranicum. For example, L. dextranicum requires glutamic acid, valine, threonine, tryptophan and histidine whereas L. mesenteroides can grow on valine and glutamic acid. Nutrient requirements for growth of the species are extensive, dextran production by viable cells is less limiting. In fact, under certain conditions polysaccharide yields are increased under limitations of bacterial growth.

The dextrans produced are polymers of glucose, joined by α -(1,6) linkages with α -(1-3) or α -(1-4) linkages at branch points. Molecular weight and degree of branching, which affect the properties of the molecule, are determined by the species and subspecies of the microorganism.

The organisms require sucrose to produce dextrans. The organism will grow on glucose as a carbohydrate source with no dextran synthesis, but production of lactic acid, ethanol and carbon dioxide can occur. Dextran production from L. mesenteroides is particularly favored at 20-25° C. Some strains, especially those from dairy sources, produce limited dextran. Slime cultures may withstand heating of 80-85° C.

The current study examined occurrence and levels of Leuconostoc spp. in cane juice in varied stages or processing in a sugar factory under normal operating conditions.

METHODS AND MATERIALS

Authentic organisms

Isolates of Leuconostoc mesenteroides (NRRL 523) and L. dextranicum (NRRC 3469), were obtained from the USDA collection at the Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture at Peoria, Illinois.

Organisms were cultured on a medium that selected for Leuconostoc and Lactobacillus (Rogosa, 1970; Rogosa et al., 1951).

A characteristic component of this medium is the presence of complex growth determinants and monooleate for maintenance of membrane integrity (Table 1). Both Leuconostoc and Lactobacilli develop on the Rogosa medium and can be differentiated by transfer to a sucrose-based medium that initiates dextran production in Leuconostoc but not in Lactobacilli.

Nutrient agar containing beef and peptone extracts was used for total bacterial counts (Buchanan, R.E., and Gibbons, N.E., 1974). Plates were incubated at 25° C for 2-3 days.

Table 1.--Composition of Rogosa medium:

		<u>Salt Solution</u>	
Trypticase	10 g	Magnesium Sulfate	11.5g/100 ml
Yeast Extract	5 g	Manganese Sulfate	2.4g/100 ml
Potassium Phosphate	6 g	Ferrous Sulfate	.68g/100 ml
Ammonium Citrate	2 g		
Glucose	20 g	Agar	5 g
Sorbitan Monooleate	1 ml	Water	1 lt
Sodium Acetate Hydrate	25 g		
Acetic Acid	1.32 ml		

Factory samples

Samples of juices were obtained from a large sugarcane factory in South Florida, in January, 1983. Weather conditions were normal with no excessively high ambient temperatures or low rainfall. Sugarcane was harvested by chopper-harvester with 12-18 in billet lengths. The burn-to-cut time ranged from 12-24 hrs. and the cut-to-crush time also varied from 12-24 hrs.

Samples were collected across the mill, of first-expressed juice (crusher juice), last-expressed juice (residual) and from the dilute juice tank (mixed juice) before screening. Juice after the DSM screens, limed juice (heated) and juice off the rotary vacuum filters were also sampled. Samples of filter cake and bagasse were taken. All samples were cooled to 5° C and transported at that temperature to Southern Regional Research Center, where they were analyzed. Total bacterial counts were determined on a nutrient agar and Leuconostoc were defined on the selective Rogosa medium.

RESULTS AND DISCUSSION

Microorganism identification

In the isolates transferred from Rogosa medium to a sucrose medium, essentially all the colonies were Leuconostoc. Tests on these using arabinose, which is fermented by L. mesenteroides but not L. dextranicum, indicated that L. mesenteroides was the predominant species (Buchanan, R.E., and Gibbons, N.E., 1974).

Two independent examinations were made of the bacterial profiles (Table 2).

Levels of both total bacteria and Leuconostoc spp. were high in first expressed juice and lower in last expressed fractions. Since a large fraction of last expressed juice was maceration water that contained no sucrose and therefore few organisms, the count was diluted. Bacteria in first juice have probably come from the field or cane yard. As a facultative anaerobe, Leuconostoc spp. can grow with or without oxygen and prefers the latter state. In sugar processing, this aerophobic property of the organism explains rapid organism growth and higher dextran levels in cane covered with mud (which keeps out oxygen) than in clean cane, and in cane stored in large piles without free oxygen circulation than in small piles or carts (Clarke, 1984).

Bacterial levels in dilute or mixed juice were higher than those in first expressed juice; this indicated that there was growth either across the mill train or in the dilute juice tank. The factory routinely applied bactericide across the second and third mills that should have inhibited microbial growth in the area. The dilute juice tank was a more probable source of contamination because it is seldom emptied completely.

Table 2.--Total bacteria and Leuconostoc population in sugar processing samples

Sample	Total bacteria x10 ⁶ /ml	<u>Leuconostoc</u> x10 ⁶ /ml	<u>Leuconostoc</u> % total bacteria
<u>First Examination:</u>			
First expressed juice	243	135	56
Last expressed juice	80	25	32
Dilute juice	290	114	39
Juice after screen	182	145	80
Limed juice	--	282	--
Juice off filter	44	.003	--
Filter cake	1	--	--
Bagasse	103	7	1
Bagasse	26	4	15
<u>Second Examination:</u>			
First expressed juice	237	163	69
Last expressed juice	84	26	31
Dilute juice	258	206	80
Juice after screen	192	109	57
Limed juice	470	240	51
Juice off filter	181	2	1
Filter cake	263	--	--
Bagasse	53	3	6
Bagasse	4	5	--

Juice after screening did not show the highest levels of bacteria; this juice represented mixed juice after "cush-cush" (bagasse/bagacillo) was screened out and before it entered the dilute juice tank where bacterial levels were higher. The results indicated that the tank was a source of contamination. The Leuconostoc fraction of total bacteria was high in the screened juice samples. The area behind the screens appeared to be the most frequent source of Leuconostoc contamination in the factory. Masses of slime material formed quickly in this area and seemed to specifically provide a source of Leuconostoc contamination and added to the bacterial levels in the dilute juice tank.

Addition of lime and heat should inhibit growth of Leuconostoc, but in both tests the highest Leuconostoc counts were observed in limed juice. Since limed juice had been expressed from the cane for a

longer period than other samples, it may be that bacterial counts increased prior to liming. Leuconostoc can grow at pH 8, but not at temperatures above 65° C; therefore, rapid inhibition of bacterial development supports the argument for hot liming, where juice is heated before it is limed. The factory where the samples were taken did not utilize hot liming, but limed the juice before heating.

It is interesting that juice samples from the rotary vacuum filters provided low levels of both total bacteria and Leuconostoc, since filters are often blamed as sources of infection. Bacteria were thought to settle out in the clarifiers with the muds and subsequently to be removed by vacuum filters. It would appear that bacteria remained on the filters with the muds but levels observed there were relatively low. Since counts represent live bacteria, it appears that clarifier temperatures were sufficiently high to kill most of the organisms in the juice and that few remained alive to inoculate samples taken after the clarifier stage. Future plans for this work include examination of samples from later stages in the factory to test this observation.

Levels of bacteria on bagasse were surprisingly low. Most bacteria must have been washed out into juice, and samples taken before there was time for a new population to develop.

The observations identify new opportunities for Leuconostoc control by competitive populations in sugar processing. Since Lactobacilli have somewhat similar properties to Leuconostoc, the possibility exists for utilization of lactics to compete with Leuconostocs in specific ecological niches. Lactobacillus cells, in their range from long and slender rods to short coccobacilli, are a bit different from spherical or lenticular Leuconostocs. Cells often occur in chains, and motility is rare. The bacteria are gram-positive with no spore formation and pigmentation is not common. Although growth of the lactobacilli can occur in air, some are strict anaerobes on isolation. Cells require complex nutritional substrates including amino acids, vitamins, nucleic acid derivatives, salts and fatty acids. Characteristically, Lactobacilli grow on carbohydrates with production of lactic acid. At least one-half of the end product carbon is lactic acid and significant pH reductions occur during the fermentation. Additional products may be acetic acid, formic acid, succinic acid, carbon dioxide or ethanol. Optimal pH is usually between 5.5-5.8 but generally cells can grow at pH 5.0 or slightly below. The primary difference between lactic acid bacteria and Leuconostoc is the absence of dextran production by the lactics. Lactic acid is not beneficial to sugar processing, but is less of a problem than dextrans.

Results of the current study also underscore some important factors in consideration of optimum time for control of undesirable

bacteria in samples during sugar cane processing. It is apparent that in the material studied the inoculum potential coming to the factory was quite high. Therefore, the problem of contamination was introduced into the initial phase of processing. Although the samples of limed juice provided high levels of bacteria, it is apparent that material at this stage of processing could be uniquely amenable to modification to achieve desired objectives. With appropriate heat treatments, the bacterial numbers could be reduced and with proper implementation of sterile practices and/or microbiocides the Leuconostoc problem could be controlled.

In addition, less expensive methods might be developed by adaptation of pH changes to development of a specific ecological niche with unique properties. Non-slime forming Lactobacilli with innocuous properties could be selected for growth in the defined niche for competitive inhibition of the Leuconostocs. We are interested in the latter approach and consider that a modest research program with pertinent objectives might provide very beneficial results.

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DISCUSSION

W. Andy Rappold, Godchaux-Henderson: Earlier you stated that in the 5.5 to 6.5 pH range the Leuconostoc did not destroy the sucrose.

Lillehoj: Oh, yes, it destroys the sucrose. It's just that you don't see any free glucose, because the Leuconostoc doesn't invert the sucrose.

Rappold: But the organism destroys the sucrose?

Lillehoj: Yes.

Rappold: Yet, when you lime further up to a high pH, the Leuconostoc increases; so therefore, would the destruction increase?

Lillehoj: I would think so. However, there is one caveat. It is clear from the literature that for the organism to produce, once it's got a population, it needs less requirement to produce the slime than it does to grow. We traditionally call growth the production of acid. So once the growing is there, it's going to produce slime. That's just the nature of the organism; it's some sort of protective mechanism, obviously. Cancellation protects the organism, so it's an advantage to it to produce slime. But you're right. That does sound like a double whammie, doesn't it?

Margaret A. Clarke, SPRI: First of all, I want to express our thanks to Dr. Lillehoj for working with us on this. We're really grateful to him for becoming interested in the problem. Your point, Andy, is very interesting to us. We had always understood, traditionally, that Leuconostoc was mostly a problem at pH's around 5 and 6. I had not realized, until Eivind had pointed it out to me, that around pH 8 was optimum for the organism, from the

microbiological point of view. I think that might explain some things that various of us have noticed over the years and have not been able to understand before, such as why we sometimes get the organisms in refineries when we think the pH is too high to allow growth.

Lillehoj: This is a very important point that's raised here. Bergey's will identify that these bugs will grow maximally around pH 5 to 6. The upper pH question is one of relative competitiveness. If you have a natural bacterial population out there and you raise the pH to 8, the Leuconostocs are going to survive. Most other bugs are just not going to compete well. Curiously, the same thing holds at the lower end of the pH range. If you drop it down to below 5, there are not many bacteria that can survive pH 5. Certainly not pH 4. And eventually, of course, they kill themselves off at the lower pH by producing acid. At both ends of the pH range, they are good competitors, but they grow maximally in the middle of the range.

Clarke: In the samples we used here, we saw amazing numbers of bacteria--they look very big to those of us who aren't used to thinking about that scale of number. Those juices used in this program were normal juices from an extremely good plant, a very good factory indeed--from good cane and bagasse. There was nothing particularly bad about the weather. It wasn't freezing; it wasn't hot. It was a normal range. Those are the sort of numbers that appear under good circumstances-- the sort of bacterial population that we're dealing with all the time.

Ronald P. DeStefano, U.S. Sugar: When you're looking at these bacteria, did you ever consider using a natural, purified, sterilized cane juice medium, rather than this more esoteric mixture that you put together, and in doing that, possibly bring out some other things that might not grow on this material, because you are looking at an undefined mixture?

Lillehoj: It's an extremely good point because, clearly, all of the requirements are in the cane. But that's a difficult kind of approach. It would require a lot of work in identifying how did it get its amino acids; what's the protease functionality--all of those factors that are going to have to be looked at. But it's certainly a good question. We didn't do that, but we certainly have talked about that. The same thing holds for lactics, for example, with grass and silage. You can't find lactics on the standing grass. It's very rare. But once you've packed that grass down with the proper moisture, in an ensiled pile, the lactics comes on like gangbusters. You don't know where they come from-- it's a very small population--but the niche is so narrow. Nothing else can survive it. For example, where is the carbon source for growing on just grass--the bug doesn't produce much cellulase. So where's the carbon coming from? There are all kinds of difficult

questions here.

DeStefano: One other thing. I think I've seen somewhere in the literature a TLC study that showed Leuconostoc working on sucrose. They did TLC at various lengths of time. They showed, first, sucrose; then I believe at some intermediate period in the middle, there was glucose and fructose. The glucose was subsequently taken up and left only fructose.

Lillehoj: Again, as I said, apparently this is not the traditional kind of conversion. You don't get a big buildup of glucose. I was interested in what Dr. Legendre had found, that there is a background of glucose, apparently, in the cane juice.

DeStefano: This was one of the things they were looking at as a possibility of picking among varieties--some have some glucose there available to work on to start with.

Lillehoj: It's a very interesting point. Obviously, there has to be some kind of effect from that glucose.

Steven A. Brooks, Barbados Sugar Research Institute: Have you used dip-slides at all in your trials?

Lillehoj: Do you mean the Gibco Company slides? No, we have not used those. For anaerobes?

Brooks: For studying the Leuconostoc in mill juice.

Lillehoj: No. We didn't use those. Do you use those?

Brooks: We tried this last year in Barbados. This area is very exciting, and we're looking at it very closely.

Lillehoj: Do they work well? Are you happy with them?

Brooks: I'm happy with the results that we've got because I have had the replications to say that they've corresponded. But not all the companies involved in this area are prepared to send people out to Barbados actually to go through the procedure. I'll be quite happy to pass any results on to you.

Lillehoj: We would like to know that, particularly if it works. It would be a nice way to look at a big population.

Clarke: I'd like to speak back to the point about glucose. We must remember that Leuconostoc is not alone in there; there are all sorts of other organisms and enzymes, including invertase enzyme, so we're always getting glucose and fructose from sucrose inverting in the cane. Leuconostoc will consume glucose alone but will not produce dextran, only lactic acid. It requires sucrose feed to

produce dextran. Leuconostoc growth helps to encourage inversion because acids form from its action that encourage inversion and make more glucose. It's not a static process, and there are other enzymes in the plant, and in the juice, that are making glucose and fructose as well.

Stanley E. Bichsel, American Crystal: We have a similar situation in the beet sugar industry. We're deluged in our diffusers with a constant level of infection of lactic acid formers, primarily. We've tried to develop means of controlling and measuring the levels of infection. In that area there are several good lactic acid analyzers out now. Ones that we're using in all our laboratories now, is the YSI unit which is fairly inexpensive. We can maintain a level of infection of about 200 parts per million lactic acid, and it only takes 3 or 4 minutes to determine that level quite accurately using the specific lactic acid probe. That might be of interest to some people here.

Lillehoj: Is that a colorimetric indicator?

Bichsel: It's an enzyme probe that YSI makes and it's specific for lactic acid. It works quite well.

Lillehoj: That would be very useful.

Bichsel: I wonder, though: What is your thinking on our control in the process? We also looked at biometers, and didn't have much luck with biometers in trying to determine the bacterial count.

Lillehoj: I suppose the best handle in the industry is the level of slime or dextrans, because that's the worry. It seems to me the place where we can manipulate the system to create the niche we want is in the liming process.

Stephen J. Clarke, Audubon Sugar Institute: I don't see any data in the clarified juice on the population of bacteria. It would be interesting to compare the clarified juice with the juice off the filter. In the clarified juice, the ratio of total bacteria to Leuconostoc is roughly 2:1, whereas in the filtrate, it's about 100:1. Are those figures comparable to clarified juice data?

Clarke: We didn't sample any clarified juice for this study. After the period of high heat in effect in the clarifier, we expected there to be lots of dead bacteria. So at this point, we don't have data on clarified juice. There would be few live bacteria left there.

FERMENTABLE SUGARS FROM STARCHES AND CELLULOSIC MATERIALS

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INTRODUCTION

Agricultural commodities have great potential as a source of nonagricultural products and chemicals (Tsao et al. 1977). Such commodities encompass agricultural residues, as well as grains, beans, wood, and sugar crops. Cereal residues make up a significant portion of agricultural residues (Detroy 1981). All of these materials will be referred to as biomass materials in this paper.

About 500 million tons of crop residues, exclusive of wood and sugar crops, are generated annually (Detroy 1981). Of this, 150 million tons are corn residue. The potential of corn residue, alone, is readily apparent when it is considered that if 10% of the corn residue were utilized, one billion gallons of ethanol could be produced, using technology available today. The importance of ethanol as an octane booster is growing, given increased demand for non-leaded gasoline additives as lead is being phased down (Anonymous 1984). Ethanol production from corn (grain) is expected to reach up to 1 billion gallons by 1985 (CAST 1984), although a subsidy is required for this octane booster to be competitively priced relative to other components such as MTBE, toluene, and tert-butyl alcohol (Anonymous 1984). Even with a subsidy, alcohol from biomass residues has not been economically attractive in the past. This has led to focusing of research activity over the last ten years to: (1) pretreatment; (2) pentose fermentation; (3) lignin utilization; and (4) energy efficient water separation from ethanol. The progress of this research is briefly summarized and analyzed in the context of fermentable sugar production costs. Advances in this area may impact the utilization of fermentable sugars for high value added fermentation products and as a substitute for molasses, before ethanol production from cellulose itself is economically feasible.

BIOMASS COMPOSITION

The major structural components of biomass are hemicellulose, cellulose and lignin at a "typical" ratio of 6:4:3 which makes up 70 to 80% of the dry matter of agricultural residues and over 90% of many hardwoods (Ladisich et al., 1984). Ample descriptions of the relationship of these components to plant cell structure are given elsewhere (Detroy 1981, and Rydholm 1965).

Complete hydrolysis of the polysaccharides encompassed by hemicellulose gives a mixture of pentoses (arabinose, xylose), hexoses (glucose, galactose), and acetic acid, with the primary component being xylose (Ladisich et al. 1984). The hydrolysis of cellulose gives glucose as the major saccharide, although degradation products made be formed if acid is used as the hydrolysis catalyst (Ladisich 1979). Lignin is a three dimensional random polymer having phenolic character which does not give saccharides upon hydrolysis. The remaining components in biomass include extractives and ash. Although these may be valuable co-products or environmentally undesirable constituents, they will not be discussed here.

BIOMASS PROCESSING

Hydrolysis of the hemicellulose component with dilute H_2SO_4 at $100^\circ C$, combined with leaching, can give primarily pentoses with few sugar degradation products (Ladisich et al. 1983). This processing step consists of: treating the biomass with 4-8% H_2SO_4 ; heating to $100^\circ C$ for 6 hours; and leaching the sugars formed to obtain a solution containing 10 to 15% sugars and 5 to 10% other components (including H_2SO_4). The material which remains is a solid and is referred to as lignocellulose.

The lignocellulose can be exposed to H_2SO_4 or cellulase enzymes to hydrolyze the cellulose to glucose. Acid hydrolysis can be carried out at 1 to 2% H_2SO_4 at 170 to $200^\circ C$. However, in this case the yield is poor (50 to 60%) with formation of significant quantities of glucose degradation products. Alternately, the lignocellulose can be steeped in 10 to 20% H_2SO_4 , filtered, dried, resuspended in water, and hydrolyzed at $100^\circ C$. The yield is 80% or higher, with little formation of degradation products. Acid and energy costs for the two processes are similar, with an independent cost analysis estimating sales prices of \$178.7 for the former and \$177.9 for the latter (Wright and d'Agincourt 1984). Estimated capital costs for these processes are also similar at \$1.28 and \$1.22 per annual gallon capacity. Research and development in the last ten years has indeed reduced projected costs. However, improvements are still needed, given the current grain alcohol price (\$1.55/gal). Research and development will lead to economically viable biomass utilization schemes. But given the large array and quantity of research results on biomass conversion, how can preliminary comparisons of hydrolysis processes be made? One attempt, based on glucose costs, is shown below.

PRELIMINARY COST COMPARISON

The major costs, exclusive of capital, are: substrate cost (S_{cost}); pretreatment cost (P_{cost}), and hydrolysis cost (E_{cost}). These parameters are given by (Ladisch et al. 1983):

$$S_{\text{cost}} = C_s / (X_{\text{cell}} \cdot Y_{\text{GE}}) \quad (1)$$

$$P_{\text{cost}} = C_p / (X_{\text{cell}} \cdot Y_{\text{GE}}) \quad (2)$$

$$E_{\text{cost}} = W_{\text{cat}} C_E / G_E \quad (3)$$

where C_s , C_p , C_E are unit substrate, pretreatment, and enzyme costs; W_{cat} is the amount of catalyst used per l of hydrolysis volume; G_E is the glucose (product) concentration in g/l; and Y_{GE} is the fractional glucose yield from cellulose. For example, let $C_s = 3$ ¢/lb (\$60/ton, dry basis); $X_{\text{cell}} = 0.4$ (cellulose fraction in substrate costing C_s); $Y_{\text{GE}} = 0.8 \times 1.11 = 0.89$ (80% conversion of cellulose to glucose corrected for 11% weight gain due to water added by hydrolysis); $C_p = 0.5$ ¢/lb (hypothetical pretreatment cost); $W_{\text{cat}} = 200$ enzyme units/l (hypothetical enzyme level); $C_E = \$20$ per 10^6 units (hypothetical enzyme cost); and $G_E = 89$ grams glucose/l $\div 454 = 0.196$ lbs glucose/l (final sugar concentration). Thus, the major costs, added together, are:

$$T_{\text{cost}} = \frac{(3 + 0.5) \text{ ¢/lb}}{(0.4)(0.89)} + \frac{(200 \text{ u/l})(0/0.002 \text{ ¢/u})}{0.196 \text{ lbs/l}}$$

$$= 9.83 + 2.04 = 11.87 \text{ ¢/lb}$$

In terms of cost, an enzyme level of 200 U/l (at $\$20 \times 10^6$ u) is equivalent to 70 g/l H_2SO_4 (as catalyst) at 2.6 ¢/lb (unit cost including acid neutralization after hydrolysis).

This particular example shows the major cost is the biomass cost. Consequently, co-product credits for pentoses (from hemicellulose hydrolysis) and lignin will be essential for reducing glucose costs. Calculations for various cases are summarized in graphical form elsewhere (Ladisch et al. 1983).

COMPARISON TO GLUCOSE FROM STARCH

The production of glucose from corn involves fractionation of starch (about 70% of corn dry weight) from the protein, oil, and fiber fractions followed by starch hydrolysis. At \$3.20/bushel corn (\$134/dry ton), the glucose cost is 8.8¢/lb and enzyme cost is 0.42¢/lb for a total of 9.2¢ (Ladisch et al. 1983; Borglum 1980). When by-product credits (equivalent to 3.2¢/lb) are subtracted, the net cost is 6.0 ¢/lb glucose. Additional credits may be realized by applying cellulose hydrolysis technology to the fiber fraction (Voloch et al. 1984). This comparison gives a goal which must be attained for glucose from biomass to be competitive to glucose from starch.

CONCLUSIONS

Biomass conversion is potentially attractive for obtaining fermentable sugars. Further research and development is needed, however. Key improvements required include: development of co-product utilization and associated marketing strategies; reduction of hydrolysis catalyst costs; and identification of biomass substrates costing \$30 to \$40/dry ton (instead of \$60 to \$70). Corn fiber (from wet-milling) may be the best candidate for a first attempt at large-scale conversion despite its high cost (\$100/ton), because of its potential for integration into existing large-scale biomass (i.e., corn) possessing facilities and associated corn product marketing strategies.

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DISCUSSION

Robert Kunin, Consultant: After your acid hydrolysis of the cellulose, you have a homogeneous base of glucose, sulfuric acid and lignin. How do you separate and recover the acid?

Ladisch: One way to separate acid from sugar is basically, the neutralization with lime to get calcium sulfate. But that's not the most economic approach. So, I would say that the fundamental aspects of our work are being published. The other aspects, perhaps, will be published at a later date. And we are now working on methods of separating the acid from the glucose itself; and there are some ways of using ion exchange resin, for example, things that have been in the literature that we are going to try now.

Stephen A. Brooks, Barbados Sugar Research Institute: Dr. Ladisch, some work was done in the Dominican Republic, sponsored by the German Government, about 5 years ago. One of the problems was at the end of the cane harvesting period. A lot of cane was unharvested, and they thought they could utilize this cane to produce alcohol. It would be the ideal situation. As far as I recall, they were going to use steam hydrolysis without any acid addition to produce the feedstock. Have you looked into this? Are you aware of this work? I am trying to get the information from the Germans. They very kindly sent it to me in German. I sent it to the German Embassy to be translated. It probably got lost between Trinidad and Barbados.

Ladisch: That process sounds similar to the so-called Stake technology and the Iotech process. Iotech and Stake processes, as far as I'm concerned, are further derived from the original technology used by The Masonex Co. They make plywood and then they make a syrup that they mix with animal feed. Basically, what they're doing there is carrying out what they call autohydrolysis. The autohydrolysis splits off the acetate groups on the hemicellulose. The temperatures will be high enough to hydrolyze most of the hemicellulose with some formation of furfural, which is undesirable, and also to hydrolyze the small portion of the glucan. So what they're trying to do there is minimize the inputs. And what they're assuming also is that the raw material--bagasse if you will--has a low value. In that case, this approach works very well. But in this country, where you can burn bagasse to generate electricity or you can burn corn residue (at least on paper), and sell back the electricity to the local power company at what they call an opportunity cost, it doesn't work so well. You have to realize that bagasse or corn residue or wood chips is worth an awful lot of money--\$60 a ton, dry weight. So what we're really looking at is a substrate, given the amount of cellulose that is present, which has very similar value to corn, when corn is at about \$2.50 a bushel. Some of these specifics with some equations, and so forth, appear in this paper.

In the last 5 years, or 7 years (I'll speak for myself), I've learned an awful lot about the complexity of the sugar industry, and how everything has to hang together. In the end when you're dealing with a renewable resource, the thing that many of us with chemical engineering training seem to forget is the fact that the resource is very, very bulky, is difficult to move, and it has to be processed when it is harvested. When you put all of these factors together, with alternate uses, you really have a very challenging problem.

The other thing I might add is that, whoever solves that problem first--how to get truly inexpensive, fermentable sugars and by truly inexpensive, I mean 5 ¢ a pound--will have a keen impact on the production of bulk and fine chemicals, using biotechnology. The markets are tremendous. There is much more than could ever be supplied by sucrose from sugarcane.

Robert F. Hutton, Consultant: You were using mineral acids for hydrolysis and that poses a problem in that you were showing a loss of glucose fairly rapidly. Is that correct?

Ladisch: Let's put it this way: I was showing that you could have a fairly rapid loss of glucose. The objective is to minimize this.

Hutton: Have you tried other sorts of acids as catalysts?

Ladisch: Yes, you can do that; the loss of glucose is less quick, but the action on the cellulose hydrolysis is also less quick. What you really have to look at is the ratio of the first constant, which is the hydrolysis of cellulose to glucose, divided by the second, which is the glucose to degradation products. The model we have here will take care of the fact that you have different acids. Again, if you look at the thermodynamic activity of the acid, that takes into account the fact that some acids ionize differently than others, and they behave differently at different temperatures. They all fall along the same line. So the real problem is to make the cellulose hydrolysis, the first step, go as quickly as possible. Because in the second step, once you pick the temperature, you're set; you're locked into a certain degradation rate, in my opinion.

Hutton: The reason for my question is that Robert Boyer of the FDA did some work on hydrolysis of the capsular polysaccharides of Pneumococci. He wanted to determine which Pneumococcus it was by profiling the mono- and disaccharides by liquid chromatography. So, it was critical to maintain the integrity of the hydrolytic products. He got, at least with those polysaccharides, relatively rapid hydrolysis with subsequent maintenance of mono- and disaccharides. And I think he's using para-toluenesulfonic acid. Is that something that will take a bite into cellulose, at any reasonable rate?

Ladisch: Yes, it will, if you are going to use temperatures at 150° C. But again, the key is, with his particular polysaccharides, they were amorphous; they did not have a crystal structure relative to cellulose. Consequently, the rate of the first step is much, much increased over the rate of the second step. The key again is: "What is it that we, as cellulose researchers, might be missing?" Lots of times we get real excited about a project; we get going into things and then we discover somebody already did it before us--maybe in 1880 or 1900.

If you compare cellulose to starch, you have to realize that starch hydrolysis with respect to enzymes really did not take off until the gelatinization of the starch was incorporated. Now starch, as it turns out, has a crystalline structure. The easy part with starch is that you can pretreat the starch and make it amorphous, so it's very reactive relative to rates of degradation, by simply heating it in water at 120° C. The trouble with cellulose is that instead of having an alpha 1-4 bond, you have a beta 1-4 bond. The hydrogen bonding of crystallinity is so strong that you don't have the option to use just plain water. As a consequence, you have to do other things.

If you, for instance, dissolve the cellulose in a solvent, (it's work that we did and lots of other people have done) and then you remove the solvent or neutralize it, and you immediately add acid or enzyme, the cellulose will hydrolyze completely. As an analytical tool, it is excellent. You don't get any degradation--you get complete glucose in 10 minutes. But from the large scale perspective, the economics really are not there yet. The key is, how do we do this in a way that optimizes the economics as well as the technology. And, I'll speak for myself again, I don't think we're there yet.

Hutton: It's a subject relatively unfamiliar to me. I understand the problem really is that you are presenting a soluble enzyme with an insoluble substrate. Can you take any heart in the fact that the opposite situation has just been revealed to be, surprisingly, a reactive one. That is to say, the interaction of soluble substrates with insoluble enzymes: that is, precipitated enzymes suspended in solution.

It is used in relatively limited cases, but it turns out that if you pick your case very carefully, for instance, esterases, you can take an enzyme, an esterase which is acetone precipitated, and suspend it in a mixture of an ester of trimyristin plus another alcohol, say, octanol, as the reactant alcohol for the transesterification. This reaction, which shows a fairly high enzymic activity, is one in which you have a precipitated enzyme suspended in two substrates. Furthermore, you can get this thing to go very actively at 100° C, because in the absence of water, you can run the enzymes at temperatures which would, normally, rapidly

denature them. It turns out that you need water for the denaturation of the enzyme, and you don't have water there either as part of the solution or part of the product.

So here is a case in which you take an insoluble enzyme, present it with soluble substrates, and get very high enzymic activities and can run them up at 100° C without denaturing them. And that, by the way, is the reason for choosing that particular reaction, a transesterification, because water is not required as a reactant nor is it produced as a product.

Ladisch: I think you've given us an example of how someone unfamiliar with an area such as cellulose hydrolysis can really cut through all the verbiage and get to the key of the problem. In fact, we now know that the cellulase enzymes act as a precipitate. What they do is literally adsorb or precipitate on the surface of the cellulose. At the surface of the cellulose, they can have a very high rate of hydrolysis. But because the cellulose is so crystalline, so tough to take apart, after a while the surface does not change greatly. Even though the local rate is very, very high, the ratio of the volume (unless you have very small particles which are expensive to get) to the surface area is fairly high as well. As a consequence, even though the local rate is high, the global rate over the particle is low.

Hutton: Is that to say that the enzyme is very effective when it first takes a bite but not after that?

Ladisch: No, it's very effective the second time it takes a bite, but the whole idea here is that the surface area, which it seeks, relative to the total volume is very small. What we are trying to do is take apart that particle so we have a tremendous surface area. We have done this technically already. Once we do this economically, that will be the key. That would be one of the keys, anyway.

The other key is byproduct utilization and market strategies. That is to say that if you get glucose cheaply from the biomass, you still have to consider that you have lignin and hemicellulose. They're worth a lot of money; you can't throw them away. You have to come up with the marketing strategy to sell these profitably well. It's a very complex business, as well as a technical proposition.

I hope I don't sound negative--I'm really very excited about this area. I'd like to come to you and say if you fund my research another 3 months, we'll be there. But in all honesty, I think it's a very complex problem. The other thing to realize is that when the payoff comes, it will be huge. I think I can cite one example which was just discussed by the Secretary of Agriculture last week. This is one where our group found out that you can use corn grits

to remove water from ethanol. You pass the water and alcohol over a huge column of corn grits and you get anhydrous alcohol. It turns out that corn also likes to store the heat of absorption. It's a good insulator. It's really inexpensive, about 10 ¢ a pound. Well, to make a long story short, the savings (the potential cost savings--this process is now in commercial operations in Ireland) is 4-5 cents per gallon of alcohol. That may not sound like very much until you realize that if half of the expansion capacity for the next 5 years uses this technology, this would mean a savings industry wide of 100 million dollars over 10 years.

It now costs \$1.80 a gallon to make alcohol, significantly less than 5 years ago. Very small incremental cost improvements may put the thing over the top, and this will have ramifications for the sugar industry.

To state another example, the corn wet milling industry makes lots of fiber-gluten feed, and ships that to Europe. Now Europe is saying, "I don't know if we want to import as much fiber anymore." That's possibly going to be brought up in the next round of trade negotiations. If E.E.C. does not accept as much fiber anymore, the wet millers will be forced to look at other uses for their fiber. This fiber contains hemicellulose and cellulose but no lignin, and this cellulose is in a more amorphous state than you find in bagasse. So wet millers may be very interested in looking at technology such as this because it would give them fermentable sugars.

If you put the fermentable sugars into your alcohol fermentation, more starch is available to make high fructose corn syrup. I think that within the next 5 years this will become a very important technology. It won't be the sort of thing where we read that someone is making 20 ¢ a pound, but rather a penny a pound. But on a large scale, it's a very significant amount of money.

I think you gave us an excellent idea here.

Leif Ramm-Schmidt, Finnish Sugar Co.: I am not really asking you a question but you said you are not so familiar with the sugar business.

In Finland, we have been doing quite a lot on the job you've just spoken about, both with hemicellulose and cellulose. With cellulose we realized, as you discussed this morning, that if you get it hydrolyzed into glucose, it will be very dilute, very impure, and so on; so it wasn't beneficial compared to very many other things, at least yet. On the other hand, with hemicellulose we have developed a commercially beneficial process. We originally used birch which was hydrolyzed by sulfuric acid, then treated by the separation that you mentioned--the one with ion exclusion in an

acidic form to get out the sugars. We get out a fairly pure xylose fraction. It is purified in a second separation, brought over to the hydrogenation stage where we convert it to xylitol. This is purified again in a third ion-exclusion separation. There are some other resin based purification steps, and carbon and so on. The final product is xylitol which is 99.9% pure. This is used instead of ordinary sugar in chewing gum in Finland and many places in Europe. It doesn't cause any dental decay and is a very good product. This production will support the regular sucrose business so that sucrose doesn't get a bad press that it's spoiling the teeth; we can say we have something else.

In this process, there are some other by-products. We have one which is called polyol liquid, which contains all the arabinose and so on which were in the hemicellulose. These may be used as raw material for different purposes. Then there is one fraction, the blackest one, the wood molasses, which is mixed in with the ordinary refinery molasses and is doing very well in the animal feed business.

Ladisch: This gives us 4 examples which illustrate the coming changes in biomass conversion. I am really interested to hear that.

Ramm-Schmidt: The raw materials for this business could be almost anything which contains enough hemicellulose. It could be bagasse also, or corn cobs, for instance, and also the husks from different grains, barley and so on, could be used. So, there are very many raw materials for that business and I suppose it will be growing. When we used birch, we used the cellulose part of it, or the hard part, for burning. We did not try to use the process on cellulose.

Margaret A. Clarke, SPRI: First I want to thank Mike Ladisch for coming here, and taking the time to spend with us at this conference. I know you're a very popular speaker and I think everyone knows why now. You've been very modest about your discovery that corn grits can be used to dry alcohol. As I recall, you also found that bagasse could be used for the same purpose. I hope we see some benefit to the industry coming out of that.

You mentioned work on glucose degradation in acids. This does have application to the sugar industry and to everybody here. One of the problems that we have in all sugar processing is decomposition of what we call invert--glucose and fructose--particularly in acid media in sugarcane factories, where you get all sorts of decomposition products coming out of the glucose breakdown. You have proposed a model for this which looks as though it goes well with the realities. It will be of considerable interest to the sugar industry.

Ladisch: Thank you for the invitation.

LABORATORY FILTERABILITY TEST METHODS

Nicholas Nenadkevich

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INTRODUCTION

The fundamental factors affecting the filtration of sugar solutions are generally considered to be pressure, temperature, filter area, filter media, viscosity and the character of the non sucrose solids in the sucrose solution to be filtered.

The significance of the filterability characteristic of raw sugar, and its effect on the cost of a plant operation is one of the most important considerations of the sugar industry. When a refinery receives a raw sugar with a poor filterability to process, it must make some type of modification to overcome this dilemma.

In view of this problem, it has become increasingly more important for refineries to have some method of predicting ahead of time the relative filterabilities of the raw sugar cargoes.

The last such attempt was the millipore membrane filtration method. In this test 300 ml of a 15°Bx of a washed raw sugar solution was filtered through a 47 mm diameter, 1.2 micron membrane with 22 inches of vacuum at 25°C for 10 min. This method failed to correlate to the actual refinery performance, for reasons, such as 1) nonuniformity of membrane porosity and 2) the test was conducted at room temperature and with a vacuum, which differ from actual refining operations.

This paper presents our efforts and success in using an alternative means to predict reliably the filterability of incoming raw sugar.

*Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

MATERIALS AND METHODS

Filtration Bomb Apparatus

The so called laboratory filtration bomb apparatus was adopted from the multiple pressure filter assembly unit developed at J. Manville Research Laboratory (Cummins and Weymouth, 1942). Basically, this apparatus "functions" similar to a refinery filter press, except its filter area cannot be precoated with filter aid prior to a filtration.

Its advantages are 1) the results obtained correlate closely to the refinery experience (Chou 1983), 2) its results can be reproduced better than $\pm 5\%$.

Filterability is defined in terms of volume of filtrate collected in 35 min at the following test conditions: 50 psi at the $80^\circ \pm 1^\circ\text{C}$, $60 \pm 0.3^\circ\text{Bx}$ using 0.35% standard laboratory diatomaceous earth and 1 in. diameter filtering area.

The apparatus consists of two individual chambers. The purpose for the two chambers is to carry out two individual tests simultaneously or to duplicate the same sample. Each chamber is 5-1/4 in. in inside diameter and 8-1/2 in. deep. Both chambers are immersed in an insulated water bath to maintain temperature at $80^\circ \pm 1^\circ\text{C}$. A layer of paraffin on the water surface is used to reduce heat loss due to evaporation. The description of the apparatus is as follows:

The water bath temperature is controlled by:

Three heat switch: 175 watt, 350 watt, 750 watt, determines output of two element heater.

Thermostat: # 17100 Hexhead 10 amp thermoswitch with modifications 6A, 85 and 13, Fenwal Inc. Ashland, Massachusetts. Controls the two element heater.

Auxiliary Heater Switch: Controls a 500 Watt auxiliary heater which is used as a supplementary heat source to accelerate initial warm-up of the unit.

Air Pressure Controller: Air pressure is provided by compressed air line that exceeds 50 lbs. and regulated by Foxboro type 67-FR control valve.

All parts coming in contact with liquid being filtered are of stainless steel. Each filter leaf is recessed to hold a backing screen with 625 holes per square inch. Each hole is .021" in diameter. The filter cloth (Victor style #407 cotton cloth) is placed over this screen and flush with the surface of the leaf. The cloth is held in place by a filter headpiece

fastened by screws to the filter base. Mechanical agitation is provided by two 1-1/2 in. diameter propellers on the shaft driven by V belt by 1/3 H.P. 470 RPM ratio motor.

RESULTS AND DISCUSSION

Parallel Evaluation of Two Similar Units

Since this type of an apparatus is not commercially available, it became necessary to see whether a new unit made to the design specifications would reproduce the same results as the old existing filtration unit. The initial test employing the procedure listed in Appendix 1 showed dissimilar results when comparing the new bomb vs. the old unit. The results are shown in Table 1.

Table 1.--Comparison of flow rates, ml/35 min, of two filtration units

<u>New Bomb</u>		<u>Old Bomb</u>		<u>%Differences</u>
<u>Left</u>	<u>Right</u>	<u>Left</u>	<u>Right</u>	
210	209*	228	223	-6.7%

*On the first try the left agitator froze, and it had to be loosened up by the manufacturer.

Percent difference is calculated by the following formula:

$$\frac{\text{Avg. Old Bomb} - \text{Avg. New Bomb}}{\text{Avg. Old Bomb}} \times 100 = \%$$

The nonuniformity of results prompted us to look for various possible causes. It became apparent that in order to have the same results with the two instruments, their main features had to be uniform.

The following parts were checked and corrected when necessary:

1. Mechanical agitation:

The new bomb's RPM were found to be different, and a pulley had to be replaced to make both units operate at 400 RPM. The test results after this modification are shown in Table 2.

Table 2.--Comparison of flow rates, ml/35 min, with equal agitation

<u>New Bomb</u>		<u>Old Bomb</u>		<u>%Differences</u>
<u>Left</u>	<u>Right</u>	<u>Left</u>	<u>Right</u>	
182	182	201	200	- 9.2
183	181	204	202	-10.3

2. Pressure Gauges

The pressure gauges of the new bomb were found graduated with 1 division equal to 5 psi, and were replaced with larger and more accurate gauges (1 division equal to 1 psi) to allow for better regulation of pressure. Flow rates after this modification are shown in Table 3.

Table 3.--Comparison of flow rates, ml/35 min, with equal pressure regulation

<u>New Bomb</u>		<u>Old Bomb</u>		<u>%Difference</u>
<u>Left</u>	<u>Right</u>	<u>Left</u>	<u>Right</u>	
191	189	218	216	-12.4

3. Backing Support Screens

The next test consisted of checking the effect of the backing screens that hold the filter cloth. The backing screens with .095 in. diameter holes were compared with backing screens with .021 in. diameter holes, and 625 holes per square inch.

Test results performed with these two varied discs with the new and the old bombs showed that the nature of the discs, the porosity, and the number of openings had no influence on flow rate. Test results showed additional indication that the new bomb had lower results vs. the old bomb. Test results are shown in Table 4.

Table 4.--Comparison of flow rates, ml/35 min, with varied backing screens

Disc Hole		New Bomb		Old Bomb		%Difference
Diameter, in.		Left	Right	Left	Right	
1.	.095	204	200	224	-	- 9.9
	.021	205	203	221	-	- 7.7
2.	.095	188	186	212	-	-11.8
	.021	189	188	210	-	-10.0

4. Filter cloth

Cotton filter cloth #407 was used for dressing the filter leaf. The effect of different purchases of these filtration cloths was studied. Test results shown in Table 5 indicated no difference when two purchases of this filter cloth were used.

Table 5.--Comparison of flow rates, ml/35 min, with different filter cloths

	New Bomb		Old Bomb		%Difference
	Left	Right	Left	Right	
Old Cloth	198	-	216	-	- 8.3
New Cloth	195	-	211	-	- 7.6

5. Filter area

It was found that the old headpieces' circular filtering areas were slightly oversized.

New Cells	(2)	found 1.000 in. diameter area
Old Cells	(2)	found 1.005 in. diameter area

The old headpieces were discontinued in the old apparatus, and were replaced with a new set of headpieces (2) with 1.000 in. diameter filtering area.

6. Hydrostatic head

Distances of filter leaves from the bottom of the New Bomb were adjusted to be equal as in the Old Bomb to approximately 1/8 in.

The New Bomb's results were still lower compared to the Old Bomb's results. At this point only the size and type of the propellers was the suspect for non-reproducible results and remained to be replaced in the New Bomb.

7. Size and shape of propeller and distance from the chambers bottom

The propeller shape and size in the new unit were found different from the old unit, and were replaced with the same (A.H. Thomas # 8295-F45).

The distance of the stirrers from the bottom of the chambers was checked and found to be equal to approximately 1". This is important, in order to keep all insolubles distributed evenly and because the degree of aeration is thought to have an effect on the filtration.

With the above items checked and corrected, the New Bomb gave comparable flow rate results to those obtained with the Old Bomb with the same sugar solutions, as shown in Table 6.

Table 6.--Comparison of flow Rates, ml/35 min, with all test parameters equal

<u>Sample Description</u>	<u>New Bomb</u>		<u>Old Bomb</u>		<u>%Difference</u>
	<u>Left</u>	<u>Right</u>	<u>Left</u>	<u>Right</u>	
Run 1 at 60 Bx	124	122	126	124	-1.60
Run 2 at 57.5 Bx	152	149	148	149	+1.35
Run 3 at 55 Bx	168	164	168	166	-0.60
Run 1 at 60 Bx	120	120	120	119	+0.42
Run 2 at 57.5 Bx	140	139	140	138	+0.36
Run 3 at 55 Bx	166	166	166	166	0.00

Percent difference is calculated by the following formula:

$$\frac{\text{Avg. Old Bomb} - \text{Avg. New Bomb}}{\text{Avg. Old Bomb}} \times 100 = \%$$

Collaborative Test With An Affiliated Laboratory

As a further check of reproducibility, the new unit was sent to an affiliated laboratory.

Thirty raw sugar cargoes tested separately by the described method, and by these two similar filtration units located at different refineries produced satisfactory results. Test results are shown in Table 7.

Table 7.—Comparison of flow Rates, ml/35 min, in an interlaboratory test of the filtration bombs

Sample #	<u>Laboratory I</u>	<u>Laboratory II</u>	Average	% Deviation of New Unit from Avg.
	(Old Unit)	(New Unit)		
1	213	216	214	0.9
2	138	143	140	2.1
3	225	222	223	0.4
4	201	213	207	2.9
5	192	205	198	3.5
6	263	264	263	.4
7	144	162	153	7.2
8	199	209	204	2.4
9	221	243	232	4.7
10	222	236	229	1.3
11	199	212	205	3.4
12	214	208	211	1.4
13	226	238	232	2.6
14	242	256	249	2.8
15	265	265	265	0.0
16	262	238	250	4.8
17	223	211	217	2.8
18	190	186	188	1.1
19	198	183	190	3.7
20	180	177	178	.6
21	216	211	214	1.4
22	222	235	228	3.1
23	195	201	198	3.5
24	188	204	196	4.1
25	263	267	265	.8
26	214	223	218	2.3
27	185	189	187	1.1
28	181	202	192	.7
29	246	218	232	6.0
30	246	218	232	6.0
Avg.	209	207	-	2.6

Based on the above results, this bomb filtration apparatus can be used in evaluating flow rates of various raw sugars, evaluation of various grades of diatomaceous earth, or experiments pertaining to any refinery filterability related problem.

The Effect of Process pH On Laboratory Filtration Rates

Regular laboratory filtration rates are performed with laboratory washed sugar liquors at various pH as described in the procedure. Filtrations were made also by adjusting the pH of the same washed sugar liquors with Ca(OH)_2 to pH 8 to reflect actual refinery operation. These sugar samples additionally were tested for amounts of P_2O_5 present.

It is noted that filtration rates of adjusted samples to pH 8 were on the average 15% slower as compared to filtration rates of unadjusted samples, as shown in Table 8. Precipitation of insoluble substances at 8 pH and blockage of the filter was the reason for the lower filtration rates.

Table 8.--Comparison of filterability of washed raw sugars at different pH

Sample #	Phosphate, PPM (as P_2O_5)		Filterability ml/35 min (ml/35 min.)	
	Raw Sugar	Washed Raw Sugar	pH Unadjusted*	pH 8
1	54	21	245	228
2	64	29	256	231
3	108	40	180	146
4	120	63	136	100
5	113	36	163	142
6	130	52	158	120
7	54	26	302	257
Avg.	92	38	206	175
% Difference	59		15	

*Unadjusted pH of the solutions ranged from 5.8 to 6.6

Filtration Effect On Turbidity

Filtered materials such as Press filtered washed sugar liquor still contain certain amounts of turbidity. This turbidity is related to the type and amount of undesirable impurities present in the unfiltered liquor as well as to the media through which this product was filtered.

Four grades of diatomaceous earths were filtered with two (2) washed sugar liquors to observe flow rates as well as turbidity of filtrates. Turbidity measurements in Nephelos units were obtained with the Coleman Model 9 Photo-Nephelometer. Effluents of the first 5 min were excluded from Nephelos determinations.

It may be noted from results in Table 9 that, as expected, the faster the flow the higher the Nephelos value.

Table 9.--Effect of grade of filter aid on filtration rates

Diatomaceous Earth Grade	Type of W.R.S. Used	Flow Rate ml/35 min	Nephelos Of Filtrate
1	1	126	23
1	2	100	23
2	1	423	43
2	2	329	47
3	1	890	66
3	2	695	73
3	1	637	55
3	2	445	53
4	1	1597	77
4	2	1376	62

Manville Walton Filter

Recently Manville Co. has introduced yet another filtration apparatus. The Walton Filter is a pressure leaf filter complete with pump, auxiliary piping and valves, all mounted on a portable stand. This filter can be operated to simulate actual production condition in the plant. A more detailed description and diagram of the apparatus can be found in the manufacturer's manual.

A procedure was developed at Operations Laboratory (see Appendix 2) by varying certain of the filter's operating parameters such as filter cake space, pump pressure, body feed concentration and time. Washed raw sugar samples tested

according to this procedure yielded results which directly correlated with the bomb filter results for the same samples, as previously reported (Chou 1983).

The correlation in filtration rate between the bomb and the newer model of Manville's Walton Filter was excellent, with a coefficient of 0.972.

SUMMARY

When a refinery receives a poor filtering raw sugar, costly remedial steps have to be taken to maintain the refinery throughput. It is for this reason that development of a filtration test method to establish the filterability of incoming raw sugar is essential in order to further reduce the refining cost.

Two filtration test methods, namely, a "bomb" test and a Manville Walton filter test were evaluated. Both methods are found to be satisfactory in correlating the filterability of raw sugar to the refinery press filtration operation. However, the laboratory filtration bomb apparatus, in our opinion, has certain advantages over the Walton filter. The bomb filter needs almost no maintenance, and it appears that it will last for many, many years. (Indefinitely). The bomb filter can be located in many less than optimum areas whereas the Walton filter requires more of an analytical or laboratory setting.

A detailed procedure for the bomb test and equipment specifications are described in Appendix 1 for those who are interested in using this tool for the control of the refining process.

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APPENDIX I

PROCEDURE FOR EVALUATION OF RAW SUGAR FILTRATION RATE

Apparatus and Materials:

1. Mixer

Kitchen-Aid model K-5, with flat beaters, # K-5-A-B, manufactured by Hobart manufacturing Company, Troy, Ohio 45374.

2. Dispensing Burette

500 ml size Corning # 2094 dispensing burette.

3. Centrifugal Machine

Model CS with 8 in. basket with draining chamber, manufactured by International Equipment Co., Heedham, MA. The inside of the 8 in. basket is to be faced with a metal screen #00 mesh 0.020 in. diameter, 625 holes/sq. in. which can be purchased from Ferguson Perforating & Wire Co., 140 Earnest Street, Providence, RI.

4. Filtration Apparatus

Laboratory Filtration Apparatus (Figure 1) from the Lipton Machine & Tool Co., 248 Devol St., Brooklyn, NY 11211.

5. Filter Cloth

42" wide Victor style # 407 cotton filter cloth available from Wm W. Stanley Co. Inc., 410 Hillside Ave., Hillside, NJ.

6. Diatomaceous Earth

Standard Laboratory Filter-Cel.

Preparation of the Affined Raw Sugar

1. Place 1000 g of well-mixed raw sugar in the mixer. Turn the mixer on to speed # 1 (low speed).
2. Gradually add 380 ml of 64.0° Bx granulated sugar syrup at room temperature. (A 64.0° Bx syrup made from high quality sugar syrup may be substituted for a syrup made from granulated sugar). The syrup is added slowly from a dispensing burette and must be added at a uniform rate during approximately 4 1/2 minutes.

3. The raw sugar and syrup continue to mix for an additional one minute. The total mixing period is 5 1/2 minutes.
4. Transfer the entire magma at once from the mixer to the laboratory centrifugal machine.
5. Bring the centrifuge up to 3000 RPM in 15 seconds and spin at 3000 RPM for exactly two minutes.
6. Remove the sugar from the basket and spread it on a clean surface in a thin layer not to exceed 1/4 inch thick.

Preparation of Washed Raw Sugar Liquor

1. Dissolve 773 g of the washed raw sugar into 516 ml of distilled water at room temperature in a stainless steel beaker (SGA B-4163-3) with variable speed stirrer. Preferably run two samples at a time.
2. 2.7 g of laboratory standard Filter-Cel is added to the surface of the liquor and allowed to settle naturally into the liquor. A long stem thermometer is placed into each beaker and it is covered with a suitable size watch glass. Subsequently these two beakers are placed on gas burners and the contents heated to 85°C without stirring.
3. The preheated liquor is stirred momentarily with thermometer and transferred one at a time with the help of a funnel to a filtration cell of the filtration apparatus also preheated to 85°C.
4. The mechanical agitator is switched on, and the assembled leaves (dressed with Victor Drill cloth, ribbed side out to the filtration cell) are positioned and screwed tight with a ratchet wrench.
5. Once the temperature in the cells reaches an equilibrium of $80 \pm 1^\circ\text{C}$ the air pressure in the bombs is then increased at the rate of 10 lb per minute for 5 min until a pressure of 50 lb is reached. The pressure is maintained at 50 lb for an additional 30 min. The filtrate is collected in a 250 ml graduated cylinder.

APPENDIX II

WASHED RAW SUGAR FILTERABILITY PROCEDURE USING THE WALTON CONSTANT RATE TEST FILTER

Equipment

Walton Constant Rate Test Filter

Laboratory Balance capacity 1000 grams, accuracy 1 gram

Timer or stop watch accurate to one minute

Two 300 ml beakers

One 600 ml beaker

Magnetic stirrer

Two graduated cylinders, 250 ml capacity (preferably accurate
to .1 ml)

Procedure

1. Adjust volume plug in cell until a 3/4 inch filter cake space is attained.
2. With all four valves in open position; close backwash valve and filtrate valve.
3. Fill a beaker with approximately 300 ml of distilled water. Position beaker under precoat recirculation tube and place pump suction tube in beaker.
4. Start pump and run at full speed until liquid comes out air vent valve, then close air vent.
5. Slow pump speed to 56% and place beaker with 0.8 gram standard earth in 250 ml of distilled water on magnetic stirrer and agitate.
6. Transfer pump suction tube to beaker containing earth under agitation and start stopwatch precoat for 5 minutes.
7. Prepare 400 ml of 50 Bx. solution from the washed raw sugar to be tested and distilled water. Filter the solution through #35 mesh Tyler screen.
8. Add 0.33 gram standard earth "admix" to the 400 ml of 50 Bx. solution, place on magnetic stirrer and agitate.
9. At the end of the 5 minute precoat cycle, open filtrate valve and close precoat valve. Transfer the pump suction tube to the sugar solution.
10. Begin recording time and volume of filtrate collected in the graduated cylinder every minute.

11. Immediately increase pump to full speed until a pressure of 20 PSI is achieved on Gauge 1.
12. Immediately decrease the pump speed and maintain 20 PSI reading on the gauge by adjusting the pump speed up or down as needed.
13. Run the filter cycle for 15 minutes.
14. A plot of the sum of the filtrate volume changes for the final 6 minutes of the test versus the filtration rates obtained from the bomb filter for a number of samples will show a direct correlation.
15. To clean the system, move volume plug to the end of the cell past the drain port. Open backwash valve and the cell air vent, and close filtrate and precoat valves. Begin circulating water through backwash line from a tap faucet until system is flushed clean.

SUGAR LIQUOR CLARIFICATION USING DIATOMITE FILTER AID

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Filtration & Minerals Technologies
Manville R&D

INTRODUCTION

Raw sugars display widely variable filtration characteristics depending upon the nature and quantity of filterable solids present. Filter aids used in sugar plants also exhibit some degree of normal variability in filtration efficiency within the finished product specifications for a given grade. These difficulties, in combination with other sources of variability, make prediction of plant scale filtration based upon laboratory testing extremely difficult. This study was undertaken to determine the particle size distributions of filterable solids in various washed raw sugars, and to measure how these particles interact with filter aids during the filtration process.

PARTICLE SIZE OF FILTERABLE SOLIDS IN WASHED RAW SUGARS (WRS)

Washed raw sugars representing very difficult filtering through very easy filtering crudes were screened to remove +20 micron particles using an electroformed screen. The particles remaining on the screen were washed, dried, and weighed. The particles which passed through the screen were dispersed in electrolyte, and tested for particle size using a Model TA Coulter Counter with a 19 um aperture. Total filterable solids were determined on each sample by dissolving 2 gm. of sugar in 100 ml. distilled water, and filtering through a prewashed and preweighed membrane (Millipore, 0.22 micron). The membrane was thoroughly washed with distilled water, dried and weighed to give the total filterable solids (FS). The Coulter Counter data, +20 micron screen residue data, and total filterable solids data were combined to give the particle size distributions shown in Fig. I.

It should be noted that the easiest filtering sugar was WRS A and the most difficult filtering sugar was WRS B. The

filterable solids in WRS B were much finer than the other sugars tested, while the filterable solids in WRS A were both coarser and lower in concentration than the other sugars tested.

The limit of resolution of the Coulter Counter with a 19 micron aperture is 0.3 microns. Particles smaller than this lower limit are not counted. Each of the curves shown in Figure I show evidence of a significant volume of particles below this threshold limit. These extremely small particles are responsible for the greatest difficulties observed in filtration.

PORE SIZE OF FILTER AIDS USED IN SUGAR CLARIFICATION

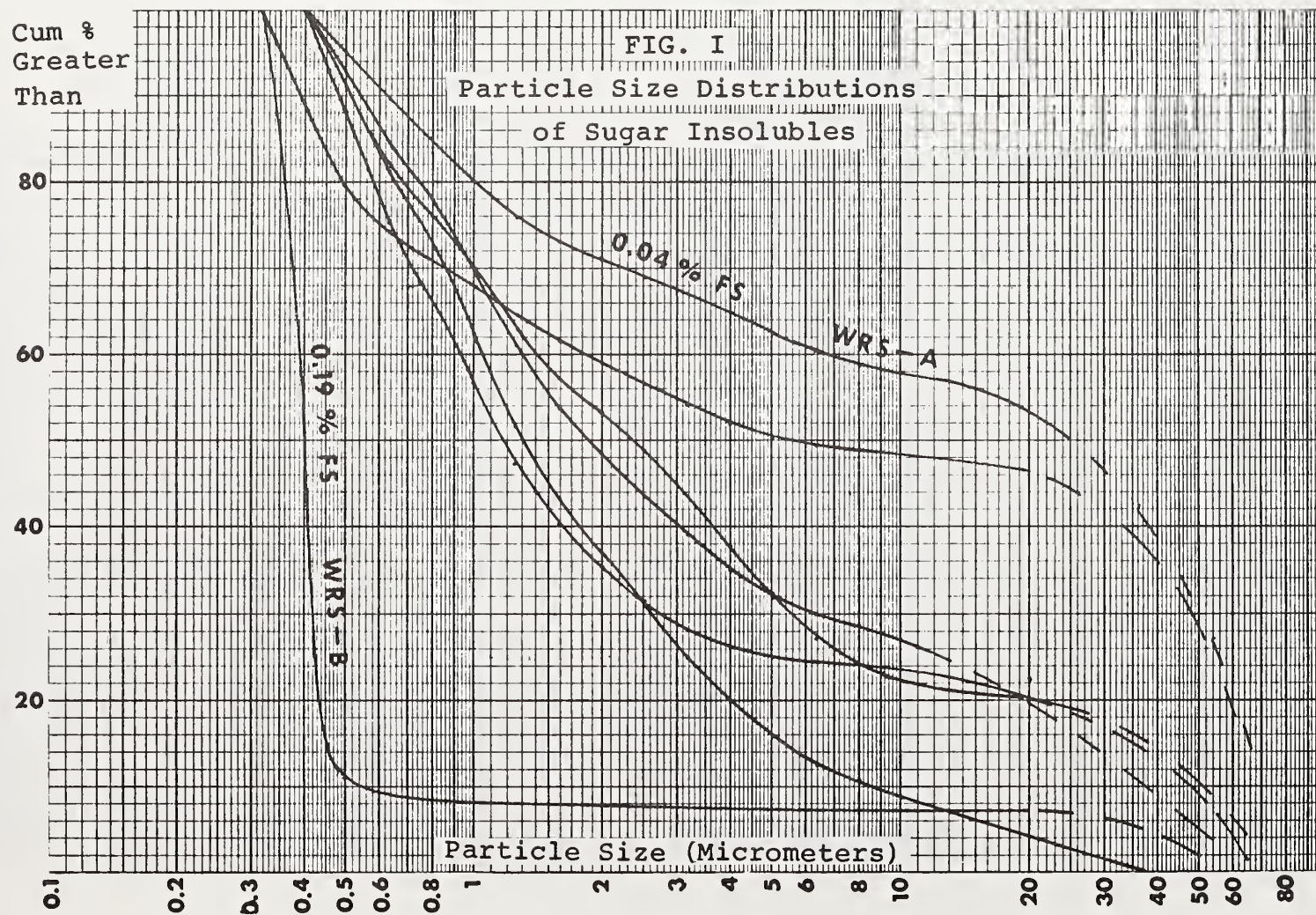
Figure II shows pore size distributions of typical filter aids commonly used in sugar clarification. Normally, best filtration results occur when the median pore size of the filter aid is equal to or somewhat smaller than the median particle size of the filterable solids. Of the seven sugars shown in Fig. I, four have median particle sizes below the slowest flow rate filter aid available. If we allow for a reasonable amount of particles less than 0.3 microns in size (which the Coulter Counter cannot detect), then only WRS A has a median particle size which can be matched by the pore size of a commercial filter aid. This mismatch of particles to pores produces some interesting difficulties which will be discussed later.

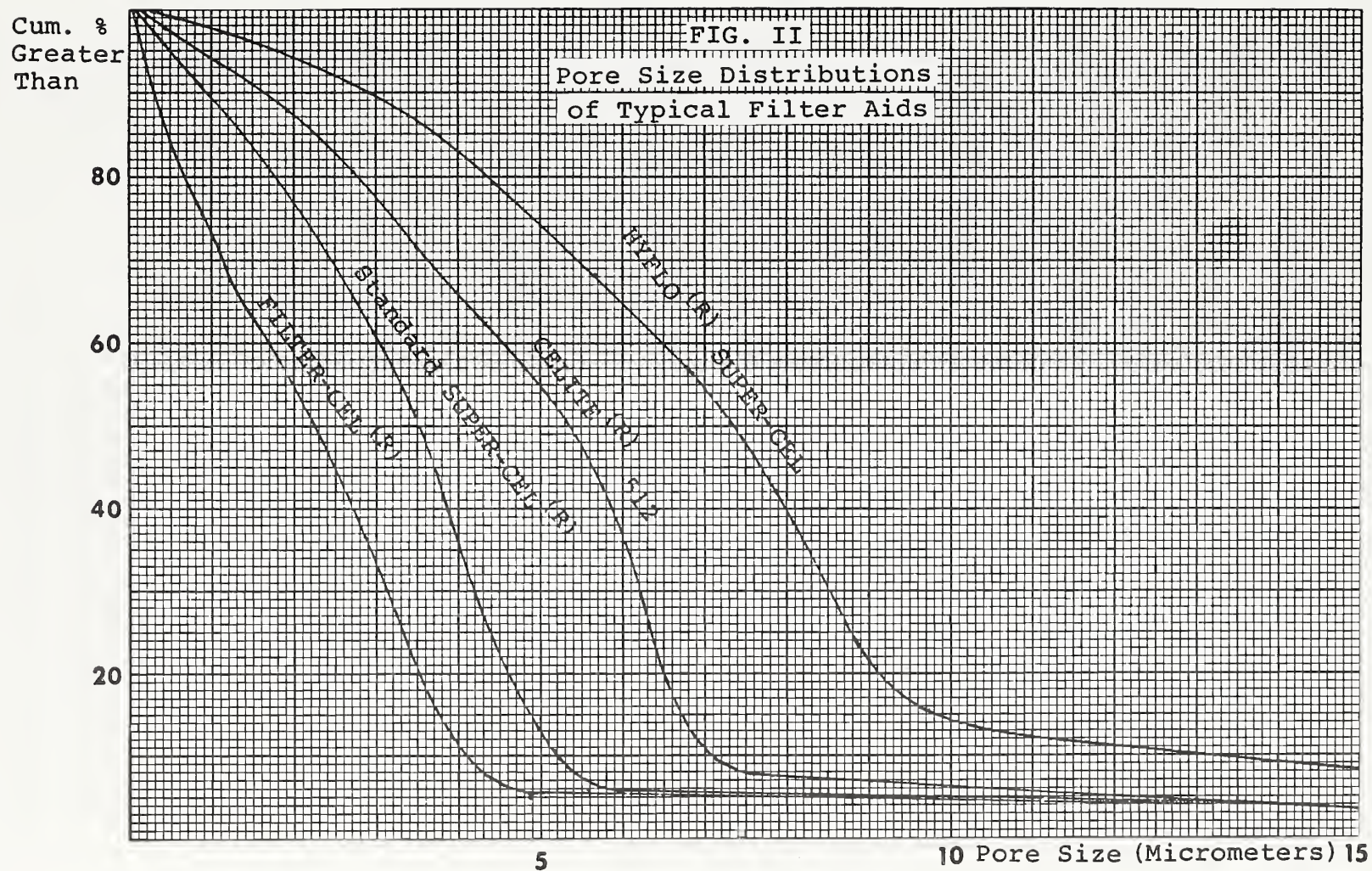
PARTICLE REMOVAL OF TYPICAL FILTER AIDS

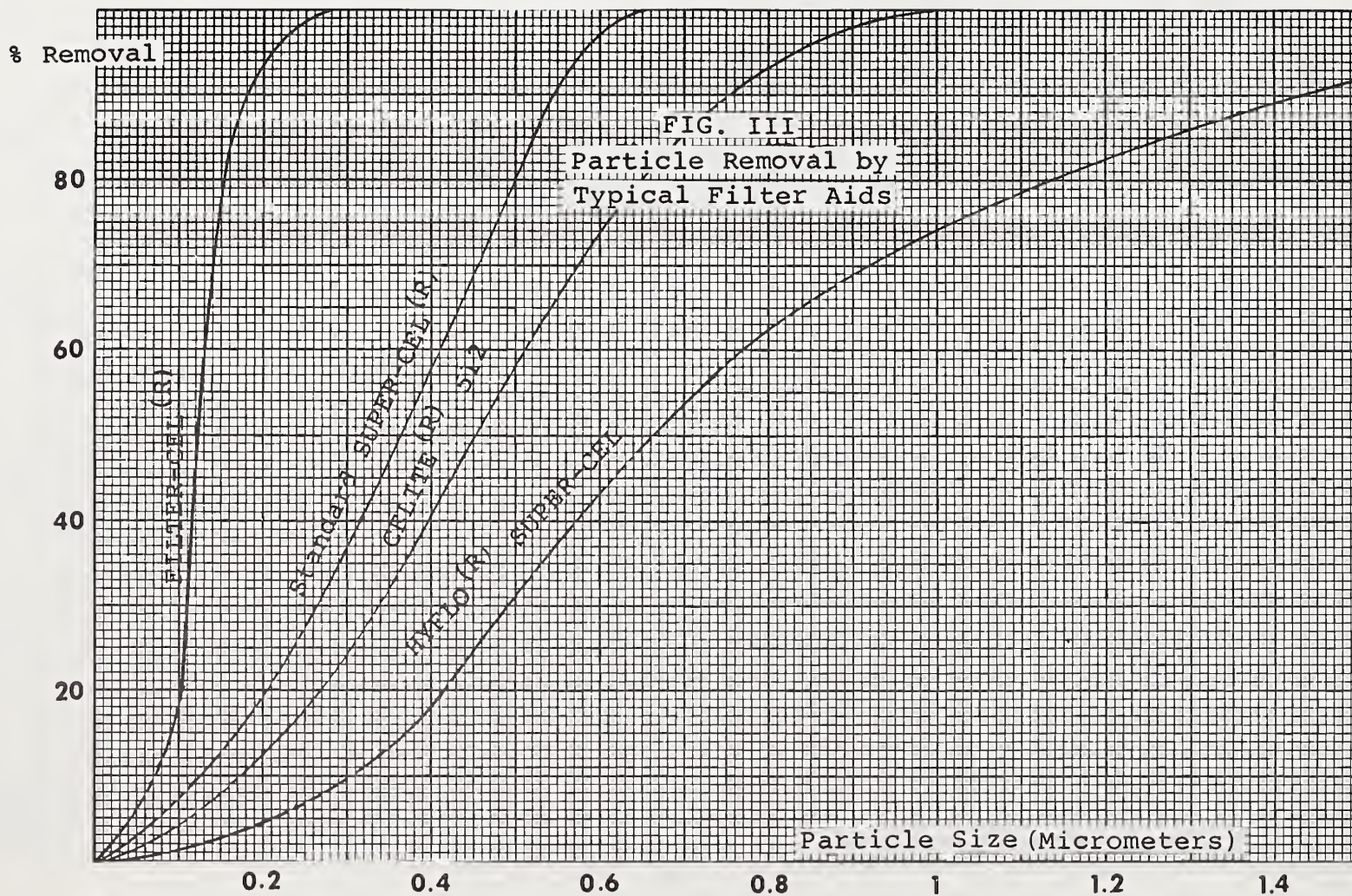
During the filtration process, a filter aid will effectively remove particles which are much smaller than the median pore size of the filter aid. Figure III shows the results of test work using closely graded silica particles suspended in distilled water. The data shown represent particle removal by a thin filter cake (0.05 to 0.10 inches) at relatively high flow rates. These curves, therefore, represent particle removal at the beginning of the filtration cycle. As cake thickness increases, and flow rate decreases, the particle removal by these filter aids will improve to some extent.

The major mechanism for particle removal by filter aids is mechanical screening. As liquid flows through layer after layer of filter aid, the effect of smaller pores becomes predominant. The particle size corresponding to 90% removal shown in Fig. III corresponds rather well to the pore size shown in Fig. II at the 95% level.

A contributing mechanism for particle removal during filtration is sedimentation. At high flow rates this contribution is very small due to viscous drag on the extremely small particles. At low flow rates, however,







the small particles can sediment in the large pores of the filter aid and restrict fluid flow.

The particle removal curves shown in Fig. III and the particle size distribution curves in Fig. I illustrate the difficult nature of sugar syrup filtration without carbonation or phosphatation to agglomerate the extremely fine particles present in even the easiest filtering sugars.

CONSTANT PRESSURE FILTRATION TESTING OF SUGAR SYRUP

Samples of WRS A and WRS B were dissolved in distilled water to give 40 Brix syrups. The syrups were filtered under the constant vacuum of 15 inches mercury using a 1.0 sq.in. septum filter area at 25 C. The filter septum used for these tests was a 110 x 20 mesh monofilament dutch weave cloth precoated with 0.5 grams of standard SUPER-CEL^(R) filter aid. Filtration data plots for the two sugars are shown in Figure IV.

Filtration theory assumes that a filter cake is formed which has constant permeability through the depth of the filter cake for an incompressible filter cake. For this case, filtration theory predicts that the filtration data plots in Fig. IV should approach a straight line having a slope of 2. We see, however, that both filtration curves shown in Fig. IV deviate substantially from predicted behavior. The sugar syrup from WRS B deviates from predicted behavior much more than WRS A.

We believe that the reason we observe this deviation in theoretical behavior is because a substantial volume of turbidity particles is smaller than the pores provided by the filter aid used. At high flow rates, these extremely fine particles are swept through the pores of the filter aid, but as flow rate decreases, the finer particles are trapped by sedimentation or bridging mechanisms. The permeability of the filter cake would therefore be expected to be higher at the septum than at the face of the filter cake.

If we assume that the permeability of the filter cake decreases as a function of flow rate, we can calculate the permeability from the filtration curves of Fig. IV using the slopes of the curves at various flow rates. Results of these calculations are shown in Fig. V. Note that the permeability for WRS B changes dramatically as a function of flow rate, while the permeability of WRS A is much less dependent on flow rate. This behavior corresponds well with the extremely fine nature of the solids present in WRS B compared to WRS A. The dependence of permeability upon flow rate for sugar filtration suggests that better

FIG. IV

Constant Pressure Filtration Data
0.5% Standard SUPER-CEL^(R) Admix

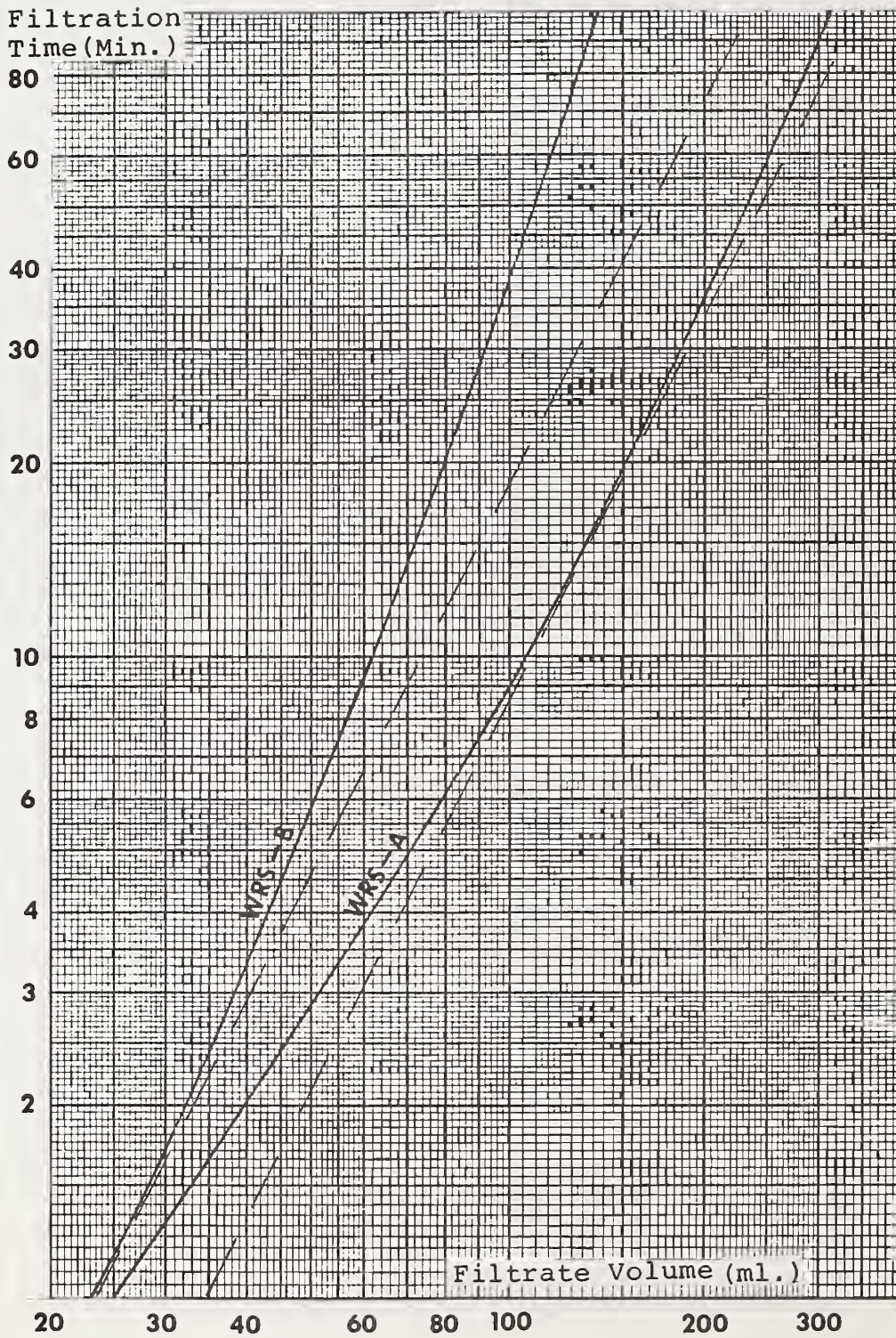
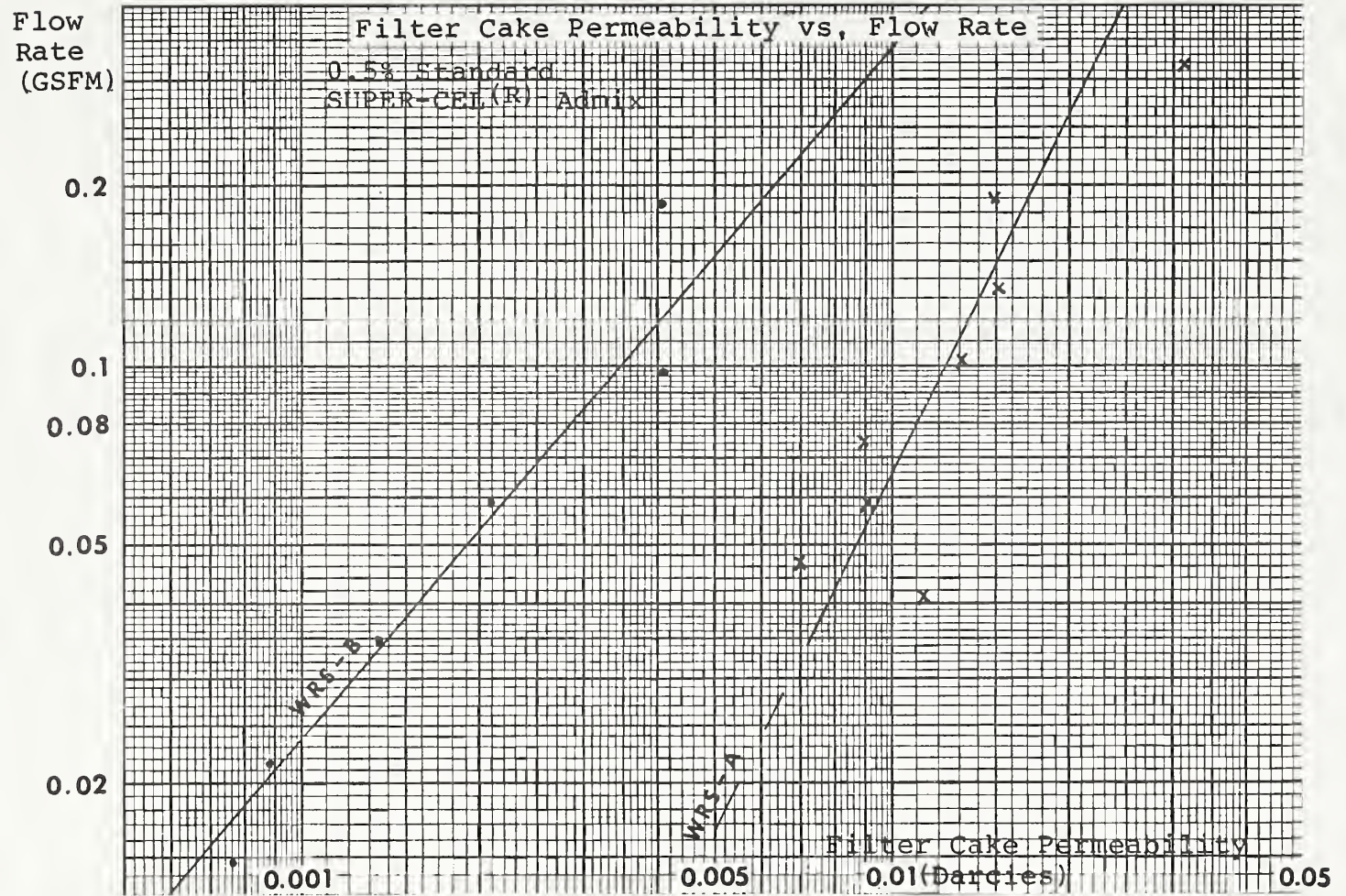


FIG. V



comparative information could be obtained from constant pressure tests if volumetric measurements were made to a limiting flow rate instead of measuring filtrate volume between given time intervals. The flow rate taken should correspond to the limiting rate at which filtration is normally stopped in the plant.

For our two examples, if we take a cut-off value of 0.04 GSFM (1.05 ml/min/in²), the test volume of liquid filtered for WRS A is 370 ml., while WRS B yielded only 90 ml. The ratio of the two values is 4.11. If we look at the volume of liquid filtered in one hour, WRS A gave 250 ml. and WRS B gave 116 ml. for a ratio of 2.16. By measuring filtrate volume to a terminal flow rate value, we improve the discriminatory power of the test by a factor of two.

DISCUSSION

Robert Kunin, Consultant: I am a relative neophyte in this area of filtration, but I do have a few questions. Did you measure the particle size at a particular Brix with your Coulter Counter? I imagine the size of many of your particles would vary with the concentration of the sugar. They are like little osmotic sponges. Were the measurements done under normal processing conditions?

Cain: No. We measured the particle size in a saline solution in the Coulter Counter, without sugar. It certainly may not represent exactly what you have in the plant or in the sugar, but it gives us an order-of-magnitude idea of what we are up against. I don't think sugar would change that greatly.

Kunin: The electrolytes that you add for the Coulter Counter might approximate the osmotic pressure of the sugar solution, so you may be getting a close idea of actual size.

Cain: We could hope that, but the thrust of the paper isn't so much particle size other than to show you the size itself that we obtained in the measurements, but to show you the problems encountered in measuring flow rates in hot pressure filtration and to give an explanation for the difficulties.

Kunin: The next question I had was: When you talk about the pore size of your filter aid, are you talking about the pore size of the particle or the pore size of the precoat itself?

Cain: It is the pore size of the precoat itself. You have pores that are internal to the Diatomite and you have pores that are between the particles of Diatomite, and the pore size distribution includes both.

Angus Cameron, Norit: You talked about the particle sizes, and you primarily talked about the nature of the particles. Do different particles of the same size filter the same way or does the physical nature of the particles also affect filtration.

Cain: I suspect we have all kinds of particles in there. You are going to hear a paper later today that indicates some of the characteristics of particles. There are a broad spectrum of particles--some are spongy and gelatinous; others are rather solid and granular. The main thing is they get stuck in the pores.

Frank G. Carpenter, U.S.D.A.: Is it the fact that you have very small particles, or is it the fact that you have a range of particle sizes that really causes filters to plug up?

Cain: That's a good question. The answer is, it's both of those reasons. Our filtration difficulties occur and begin to get serious when the particles get below a half micron size. Particles that are less than a half micron size, even if they have narrow size distribution, are difficult to filter.

QUANTIFICATION OF THE EFFECTS OF DIFFERENT RAW SUGAR IMPURITIES ON FILTRATION RATES IN CARBONATATION REFINERIES

Peter Hidi and Robert J. McCowage

CSR Limited

INTRODUCTION

In the introduction to his paper presented at the 1983 meeting of the SIT Chou (1983) stated that :

"a quantum jump in energy cost in the last decade makes it imperative, (1) to evaluate and to quantify the effects of poor filtering raw sugar on refining operations, (2) to identify the filtration impeding impurities and (3) to explore unconventional remedial steps to minimise the problem".

Similar conclusions had been drawn in the Australian sugar industry and in the early 1980's steps were taken to address the second of these points. The work focused on carbonatation refineries and also sought to quantify the effects of the different impurities on filtration rates.

It was decided that a fundamental approach to the problem was required. Hertzberg and Mountfort (1959) established the usefulness of small scale carbonatation tests for studying refinery carbonatation and filtration some 25 years ago and this was the approach used.

Of course a number of similarly based studies have appeared in the literature (Koga et al 1966, Dawes 1967, Bennett and Gardiner 1967 and 1969, Murray 1972 and 1974). Some early studies suffered from the fact that the filtration rates of the laboratory slurries differed markedly from those of actual refineries. This improved in later work but often just one compound was studied, starch being a common subject in the late 1960's, or only general correlations with measured impurities were attempted. Often conflicting findings were reported. While starch, gums, waxes,

phosphates, silicates and proteins have been identified as being associated with filtration impedance the subject is not well understood.

THEORY AND DISCUSSION

The fundamental Carman equation for any filtration is :

$$\frac{d\theta}{dV} = \frac{\eta r c V}{P A^2} + \frac{\eta R}{P A} \quad (1)$$

where θ = time from application of pressure (sec)
 V = volume of filtration in time (cm^3)
 η = viscosity of filtrate (poise)
 P = pressure difference across the cake and septum (dynes per cm^2)
 A = cross-sectional area of cake (cm^2)
 c = concentration of mud in the slurry (gm per cm^3)
 r = the "specific resistance" of the cake (cm per gm)
 R = the initial resistance of the septum (cm)

The specific cake resistance, r , is not an ideal practical measure of filtrability since it does not include the important factors of density, viscosity, and insoluble solids concentration. Hertzberg and Mountfort (1959) evolved the term slurry resistance, S , to account for this. S is given by the relationship :

$$S = \frac{\eta r c}{b^2} = \frac{\eta r c 100^2}{\rho^2 Bx^2} \quad (2)$$

where b = concentration of solute in filtrate (gm per cm^3)
 ρ = density of filtrate (gm per cm^3)
 Bx = brix of filtrate

For constant pressure filtrations the slurry resistance of a carbonatated liquor can be determine from a straight line plot of θ/V against V . The slope of this line is the group $\frac{\eta r c}{2 P A^2}$, so S can be calculated from:

$$S = \frac{2P A^2 m 100^2}{\rho^2 Bx^2} \quad (3)$$

where m = slope of the θ/V against V line

No general quantitative relationship between impurity content and slurry resistance could be found in the literature. Such a relationship was desirable so that the effect of different impurity fractions could be quantified.

It is apparent from equation (3) that with constant brix, filtration pressure, and filtration area the slurry resistance is proportional to the inverse square of the filtrate volume. Thus, using the filtrability index approach (Bennett 1967), a carbonatated filtrability, F , which is directly proportional to the filtrate volume can be defined :

$$F = \frac{k}{\sqrt{S}} \quad (4)$$

Selecting a value of 2×10^5 for k gives values of F which, for very bad sugars, approach zero and for impurity free sugar, is approximately one hundred. This is a very useful range for conceptualising the carbonatated filtrability results.

Nicholson, Hidi and McIntyre (1961) showed that with the Nicholson and Horseley (1956) filtrability test the logarithm of the filtrability of an impure/pure sugar mixture was linearly related to the proportion of impure sugar in the mixture. A filtration impeding activity, fia , concept was developed where :

$$fia = -\log \frac{\text{filtrability}}{100} \quad (5)$$

In most cases the filtration rate of a mixture of raw sugar solutions could be simply calculated by adding the filtration impeding activity of the components according to the percentage they contributed to the mixture. The range of applicability and an explanation of this logarithmic relationship was discussed by Sutherland (1966) and Hidi (1966).

The applicability of this approach to carbonatation filtration was investigated by mixing different combinations of good and bad filtering sugars, carrying out standardised small scale carbonatations and measuring the slurry resistances of different combinations. When the slurry resistances or carbonatated filtrabilities were plotted as a function of the mixture composition a straight line was not obtained. However, as with the earlier work, a linear function was obtained when $-\log \frac{F}{100}$ was plotted against the

percentage of bad filtering sugar in the mixture. This relationship was tested with seven different mixtures of sugars of different impurity compositions and was found valid in the $0.6-40 \times 10^7$ slurry resistance (10-80 carbonatated filtrability) range. As will become evident later in this paper, it also applies to the effect of most added impurities over a wide concentration range.

EXPERIMENTAL

Small scale affination and carbonatation procedures were developed specifically for these studies. The most important requirement was to reliably duplicate refinery filtration performance on kilogram quantities of sugar.

Affination

This was a two stage procedure using a 200 mm diameter laboratory centrifuge to affine to 0.1% reducing sugars content. Firstly a standard affination was performed in which 1 kg of sugar was mixed with 400g of a 65° Brix solution, prepared from the same sugar, and centrifuged at 1000 rpm for 7 minutes. This was followed by the affination of a second sample in which the amount of syrup used was selected in accordance with a simple formula based on the standard affination result and the affination target.

Carbonatation

The carbonatation equipment is shown pictorially in Figure 1 and schematically in Figure 2. It consists of a 1 L conical glass container with inlets for gas, lime, water, pH electrode, thermometer stirrer and sampling. It is held at 80°C in a thermostatically controlled water bath. The gas supply can be taken directly from the pipe supplying the refinery carbonatator or supplied independently from a mix of carbon dioxide, nitrogen and air of similar composition to refinery flue gas.

The lime supply (8° Brix) is kept in suspension by stirring and continual recirculation using a variable speed peristaltic pump. A second pump feeds the lime to the carbonatator from the recirculation line.

Liquor (64° Brix) and lime are fed to the carbonatation vessel to achieve a lime dosage of 0.50%. Approximately 800 mL of liquor is carbonatated in each run. The degree of carbonatation is controlled by regulating the flue gas supply using a magnetic gas valve. This valve is operated automatically by a pre-set Radiometer 28 pH meter connected to a Radiometer II titrator guided by the pH in the carbonatator. When the pH in the carbonatator, as measured

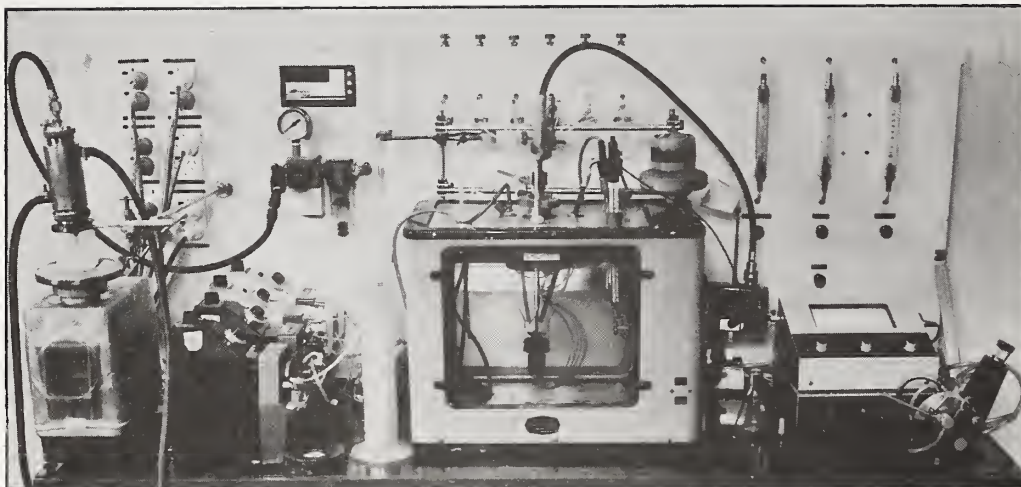


Figure 1.--Small scale carbonatation rig

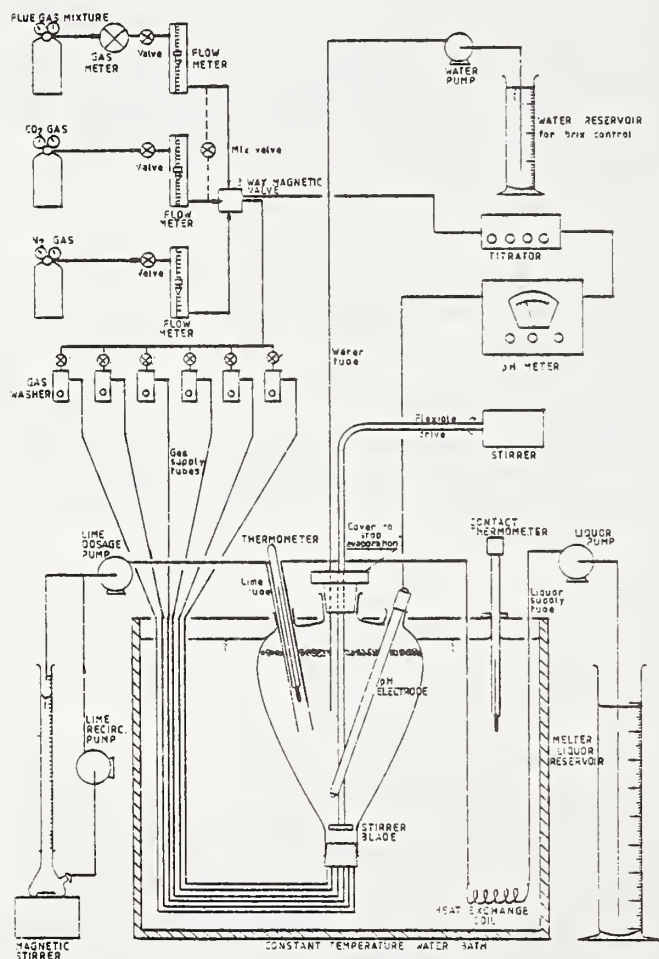


Figure 2.--Schematic of small scale carbonatation apparatus

by a Sensorex S300c glass-calomel pH electrode, drops below the selected value the flue gas stream is cut-off and simultaneously replaced by a similar rate of nitrogen gas coming through the other side of the three-way magnetic valve.

At the start of each run the carbonatator is seeded with 70 mL of refinery carbonatated liquor. This was found necessary to reproduce refinery performance. The carbonatation then proceeds in two stages. The first stage takes about 1 hour, when approximately 800 mL of affined sugar liquor and the corresponding amount of lime slurry have been carbonatated. During this stage the pH of the carbonatator is controlled at pH 8.7 to hold the degree of carbonatation at approximately 92%. In the second stage the pH is set to 7.7 and the degree of carbonatation rises to approximately 99%.

Filtration

Filtration rate and slurry resistance measurements are carried out using a 200 mL jacketed millipore filter body held at 80°C. Whatman No. 1 filter paper is used as the filter septum. The filtration area is 13.85 cm² and the applied pressure 340 kPa. Filtrate weight is recorded at 15 second intervals and the slurry resistance calculated according to equation (3).

Comparison with Factory Slurries

The slurry resistance of liquors carbonatated in the refinery was compared with those obtained in the laboratory. Brix and/or temperature correction of refinery data was usually necessary to align with the standard conditions of the small scale procedure. The success achieved with the small scale carbonatator can be appreciated from the data presented in Figure 3 which compares its results to those obtained in the factory with identical sugars and lime slurries.

IMPURITY ADDITIONS

The first approach used in these studies was to add impurities known to be present in raw sugars to solutions prepared from affined sugar of good filtering quality. The solutions were then carbonatated and the fia of the impurity calculated. All compounds were tested in several concentrations including those estimated for a typical raw sugar and concentrations about an order of magnitude higher.

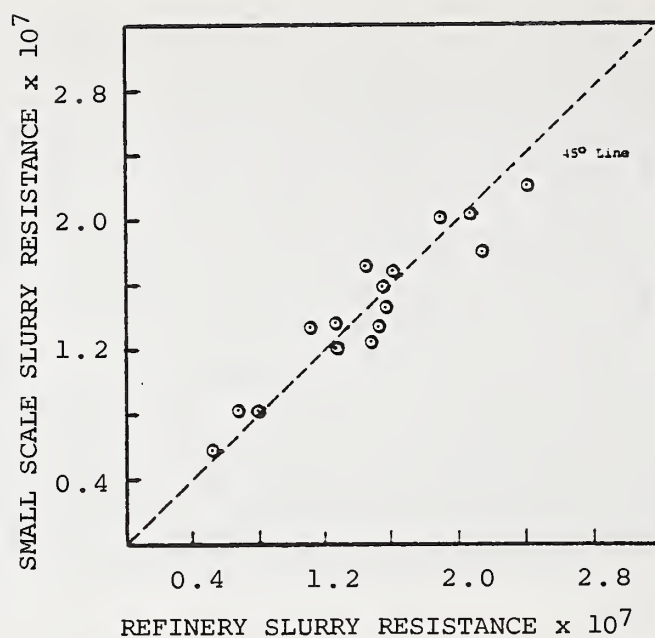


Figure 3.--Comparison of small scale and factory slurries

Soluble Impurities

There were a number of organic and inorganic soluble compounds tested which had no affect on carbonatated filtrability. These compounds are listed in Table 1.

Table 1.--Soluble impurities found to have no significant effect on carbonatated liquor filtrability

Impurity	Highest Concentration Applied (g/100 g solid)
KCl	0.5
NaNO ₃	0.1
NaCl	0.2
K ₂ SO ₄	0.2
CaCl ₂	0.15
FeCl ₂	0.05
Ammonium acetate	0.1
Sodium lactate	0.3
Sodium citrate	0.3
Sodium aconitate	0.4
Asparagine	0.15
Aspartic acid	0.05
Glycine	0.1

There are other groups of soluble materials such as hydrolysed starches and low molecular weight dextrans which also have no effect on carbonatated filtrability. These are discussed with their higher molecular weight counterparts in a later section.

Soluble Phosphate. The effect of soluble phosphate was determined by adding KH_2PO_4 to affined liquors prior to carbonatation. Data are presented in Table 2.

Table 2.--The effect of soluble phosphate on filtration properties

KH_2PO_4 added (Mg P/kg)	Slurry Resistance ($\times 10^{-7}$)	Carbonatated Filtrability	Fia
Control	0.54	86	0.066
24	1.81	47	0.327
48	4.96	28	0.547
72	12.30	18	0.745

A linear relationship was found between fia and phosphate content as shown in Figure 4. The filtration impedance arises because of the formation of a fine calcium phosphate precipitate. The fia per 100 mg/kg of soluble phosphate (expressed as P) is 0.96.

Soluble Silicates. Soluble silicates in the form of a Na_2SiO_3 solution were similarly tested. Data are presented in Table 3. Again a linear relationship was found between fia and soluble silicate concentration (expressed as Si) with the fia per 100 mg/kg of soluble silicate being 0.45.

Table 3.--Effect of soluble silica on the filtration properties

Na_2SiO_3 added (mg Si/kg Solid)	Slurry Resistance ($\times 10^{-7}$)	Carbonatated Filtrability	Fia
Control	1.53	51	0.292
22	2.27	42	0.377
74	8.26	22	0.657

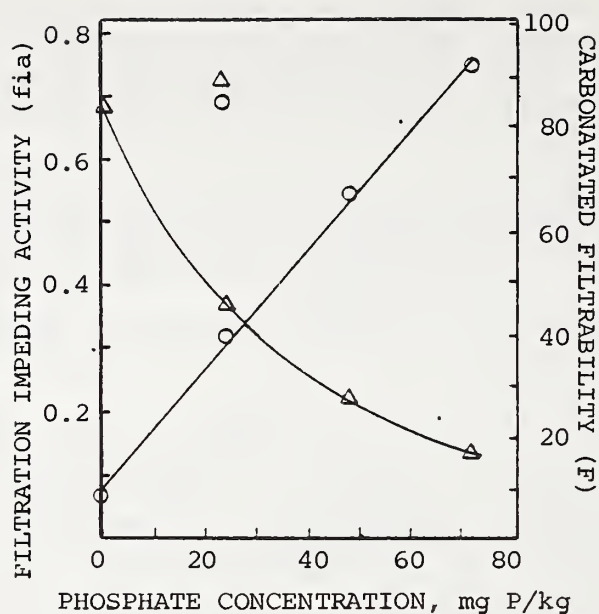


Figure 4.--Effect of soluble phosphate addition in filtration rates

Aluminium Salts. Aluminium ions were added in the form of an AlCl_3 solution in concentrations up to 200 mg Al/kg. Data are presented in Table 4. The fia per 100 mg/kg Al was 0.40.

Table 4.--Effect of soluble aluminium salt addition on filtration properties

AlCl_3 added (mg Al^{3+} /kg Solid)	Slurry Resistance ($\times 10^{-7}$)	Carbonated Filtrability	Fia
Control	1.01	63	0.201
5	1.04	62	0.207
20	1.54	51	0.294
100	6.40	25	0.641
200	27.78	12	0.921

Magnesium Compounds. Magnesium was added in the form of a MgCl_2 solution. A linear relationship between fia and magnesium concentration was not found. Up to approximately 100 mg/kg Mg had negligible effects. Beyond that concentration the effect increased rapidly. Results are presented in Table 5 and Figure 5. It can also be seen that the reproducibility of the carbonation was poor in the

presence of elevated magnesium concentrations. It is felt that the filtration impedance of magnesium is associated with the formation of a fine magnesium oxide precipitate. The non-linear results obtained with magnesium addition may be caused by the low, but not negligible, solubility of MgO in hot sugar solutions in the 7-9 pH range.

Table 5.--Effect of soluble magnesium salt addition on filtration properties

MgCl ₂ added (mg Mg ²⁺ /kg Solid)	Slurry Resistance (x 10 ⁻⁷)	Carbonatated Filtrability	Fia
Control	1.01	63	0.201
80	1.19	58	0.239
110	1.04	62	0.208
125	1.54	51	0.296
160	8.26	22	0.658
185	5.49	27	0.570
300	15.62	16	0.791
375	7.56	23	0.637
500	9.07	21	0.686
500	27.8	12	0.927

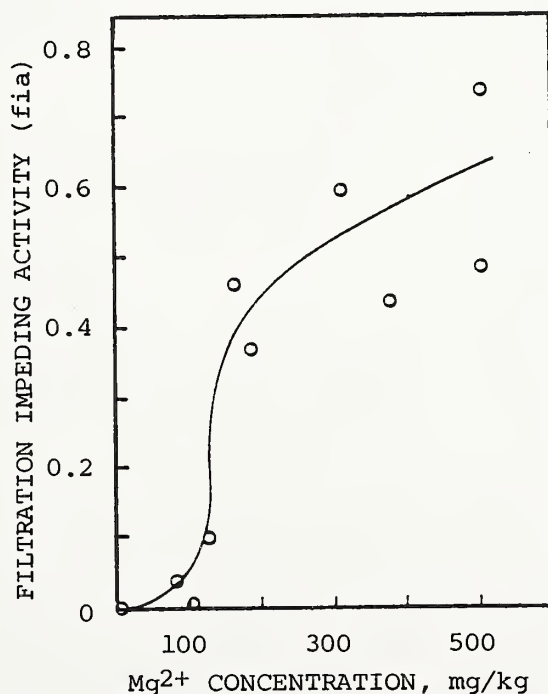


Figure 5.--Effect of magnesium chloride addition on filtration rates

Colloidal Impurities

The amount of colloidal impurities in raw sugars is small, depending on the quality of the raw sugar itself. Two groups of colloidal compounds were considered. The first group included macromolecular compounds such as starches, dextrans and gums. The second group was finely dispersed 0.3-0.4 micron inorganic compounds.

Starch. Cane starch separated from NCo 310 cane was available for testing. A 1% aqueous solution was gelatinised and added to a good filtering sugar in 200, 400 and 600 mg/kg concentrations. The effect of commercial (Univar) soluble starch was also measured. Results are shown in Figure 6.

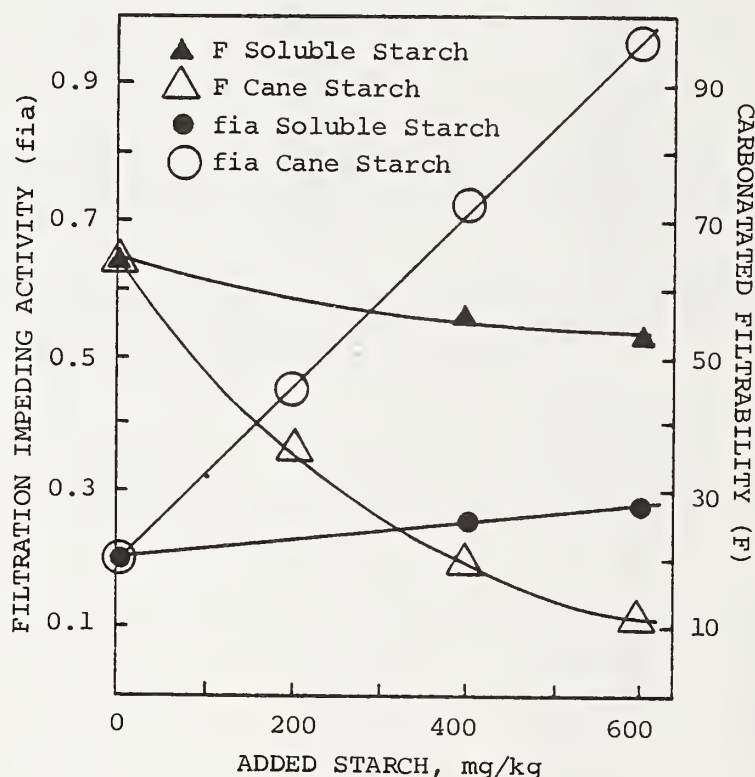


Figure 6.--Effect of starch addition on filtration rates

Starch was found to play an important role in carbonatated filtrability however the degree of starch hydrolysis (molecular weight) is also very important. The fia of soluble starch is an order of magnitude less than that of

cane starch. Similar results were obtained with commercially available wheat and corn starches in a natural and partly hydrolysed state. The molecular weight dependence of the filtration impeding effect of starches is important as starch in raw sugars is expected to cover a wide range of molecular weights due to the action of natural and added enzymes on the cane starch molecules. Thus raw sugars with apparently similar starch content may not necessarily give similar performance during processing.

These findings are supported by trials carried out at a CSR refinery using enzyme to reduce starch levels in poor filtering raws in the early 1960's (Burgess, Hidi and Holdgate 1966). In one trial the addition of Super Rapidase at 100 mg/kg sucrose resulted in the reduction of starch from 390 mg/kg to 210 mg/kg. This gave a seven-fold increase in filtrate volume per cycle of pressing. The relatively large increase in filtrate volume was not fully understood at the time.

Table 6.--Effect of 100 mg/kg of different starches on filtration properties

(The fia_{100} values were obtained by linear interpolation from several fia data)

Impurity Added	Slurry Resistance ($\times 10^{-7}$)	Carbonatated Filtrability	fia_{100}
Control	1.01	63	0.201
Cane starch	1.81	47	0.201 + 0.13
Wheat starch	1.70	49	0.201 + 0.11
Corn starch	1.81	47	0.201 + 0.13
Soluble starch	1.07	61	0.201 + 0.013
Partly hydrolysed wheat starch	1.07	61	0.201 + 0.017
Partly hydrolysed corn starch	1.07	61	0.201 + 0.014

Dextran. Partly hydrolysed dextrans with different apparent molecular weight are commercially available. The effect of three of these on carbonatated filtrability was tested and it was found that samples with molecular weight no larger than two million had no significant effect. On the other hand, dextran with molecular weight stated to be $5-40 \times 10^6$ had very serious effects as can be seen from Figure 7. All dextrans were tested up to 1000 mg/kg. Results are summarised in Table 7.

The molecular weight dependance of the dextran effect was further demonstrated by dextranase treatment of a very high MW dextran solution. With mild dextranase treatment, which cut the large dextran molecules randomly and reduced the apparent concentration by 24 percent, 82 percent of the dextran related fia was removed.

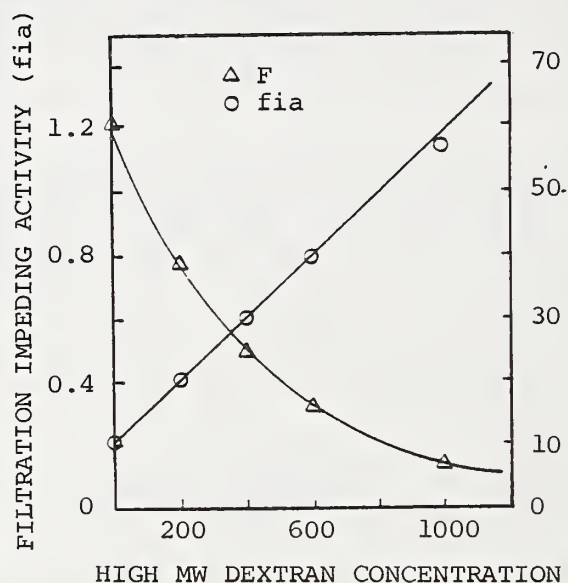


Figure 7.--Effect of high molecular weight dextran (5-40x10⁶ MW) addition on filtration rates

Table 7.--Effect of different dextrans on filtration properties

Impurity Added	Dextran Added mg/kg	Slurry Resist. (x 10 ⁻⁷)	Carbon. Filt.	Fia of 100 mg/kg
Control	-	1.01	63	-
Commercial Dextran MW 0.11 x 10 ⁶	1000	1.01	63	-
Commercial Dextran MW 2 x 10 ⁶	1000	1.01	63	-
Commercial Dextran MW 10 ⁷	1000	81.6	7	0.095
Dextran ex Mill Syrup	1000	40.0	10	0.100
Dextran ex Raw Sugar	1000	10.0	20	0.070

Gums. In contrast with starch and dextran, which are built exclusively from glucose units, the hydrolysis of gums show that they can also contain arabinose, galactose mannose, rhamnose and xylose. Very little has been published about their chemical structure or concentration in raw sugars.

The effect of gums was evaluated by testing the effect of several commercially available gums and a gum separated from a sugar mill syrup stream after starch and dextran removal.

Results are presented in Table 8. These indicate that gums are likely to cause significant filtration impedance although more information about the nature and levels of gums in raw and affined sugars would be required before definite conclusions could be made.

Table 8.--Effect of different gums on filtration properties

Gum Added	Conc. mg/kg Solid	Slurry Resistance ($\times 10^{-7}$)	Carbonatated Filtrability	Fia
Control	-	1.01	63	0.201
Locust Beam	100	2.62	39	0.409
	500	250.00	4	1.398
Karaja	500	9.07	21	0.678
Carragenan	250	1.74	48	0.319
Ex Factory Syrup (Amylase and Dextranase treated)	500	2.38	41	0.387

Colloidal Inorganics. Aluminium and magnesium oxide powders in particle sizes less than 5 microns were added to sugar solutions with the expectation that they would form colloidal solutions. However, due apparently to the hydrophobic surface of the powders and the high viscosity of the 64° Brix sugar solutions, it was found that the powders did not disperse readily even after high speed stirring. The agglomerates formed fell into the class of insoluble impurities. Results were not reproducible.

Although colloidal silica was not added to liquors prior to carbonatation it is believed that it has an important effect. It is known that colloidal silica can be readily solubilised at temperatures of 70 to 100°C and pH's in the 8-9 range. These conditions are similar to the conditions of the sugar solution during carbonatation.

A test was carried out on sugar solutions held at pH 8 and 80°C for one hour. The soluble silica content increased from 5 to 48 mg/kg. The fia of the colloidal material is thus expected to be similar to that of soluble silica.

Coarse Insoluble Impurities

A coarse fraction, which contained material greater than 8 microns in particle size was separated from a bulk quantity of raw sugar. This consisted of heavy particles and fibre with a small amount of colloidal material trapped within it. When added to a good filtering sugar solution prior to carbonatation in ten times its original concentration only a moderate decrease in carbonatated filtrability resulted. The fia of the material was found to be 0.02 units per 100 mg/kg.

Comparison of Different Impurities

A summary of the significant results of the impurity addition trials are presented in Table 9.

FRACTIONATION STUDIES

Raw and affined sugars were fractionated using two different approaches in an attempt to confirm the findings of the impurity addition trials.

Membrane Filtration

In the first approach membrane filtration was used to fractionate the impurities according to their apparent molecular size. The following three fractions were prepared initially :

- . insoluble fraction (particles retained by a Millipore membrane filter with 8 micron pore opening).
- . colloidal fraction (material passing the 8 micron membrane but retained by a analysis membrane having an estimated cut-off point in the $10-20 \times 10^3$ dalton molecular weight range).
- . soluble fraction (containing impurities with molecular weight less than $10-20 \times 10^3$ dalton).

Table 9.--Effect of different impurities added in 100 mg/kg concentration on the filtration rates of carbonatated slurries

Compound	Filtration Impeding Activity (fia per 100 mg/kg)
Soluble phosphate (as P) Soluble silicate (as Si) Soluble aluminium	0.96 0.45 0.40
Soluble magnesium (a) (b)	0.04 0.46
Cane starch Soluble starch	0.13 0.02
Dextran	
MW 40,000	0.00
MW 100,000	0.00
MW 2,000,000	0.00
MW 4-50,000,000	0.10
Gum (ex raw sugar) Course insoluble	0.19 0.02

(a) up to 100 mg/kg
(b) above 150 mg/kg

Four sugars were selected and the fia of each fraction was determined. These are combined in Figure 8 to show the relative contribution of each to the overall filtering quality of the sugars. All sugars are Australian raws. These are low soluble phosphate, low soluble silicate but moderate colloidal silicate sugars.

When further separation of the colloidal impurities to different subfractions was carried out the results were not reproducible. This is believed to be due to activity losses occurring in the isolated colloidal fraction during these operations and also during storage. The loss of activity of the destabilised colloidal systems is not unusual however it did considerably restrict the impurity separation studies.

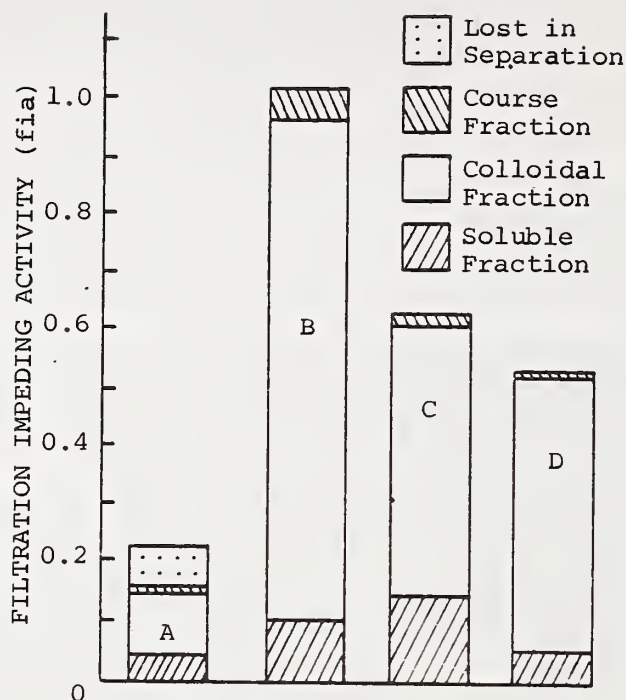


Figure 8.--Effect of different sugar impurity fractions on filtration rates

Centrifugation

Ultracentrifugation was also employed to study the role of the colloidal fraction. By centrifuging at very high speed it was possible to remove about 90 percent of the colloidal impurities. Furthermore, by varying the centrifugal speed, it was possible to change the amount of colloids removed. Combining ultracentrifugation at different speeds and small scale carbonatation of the supernatant, it was possible to assess the importance of the colloidal fraction. The effect of different centrifugal force on the fia of one raw sugar is shown in Figure 9. It should be noted that the coarse impurity fraction was removed from the liquor before centrifugation.

The ultracentrifugation approach was subsequently applied to a random selection of fourteen Australian raw sugars. Syrups of 45°Bx were sieved through a 400 mesh screen to remove particles greater than 38 microns and then they were ultracentrifuged at about $1.8 \times 10^4 g$ for one hour. The supernatants were analysed before and after for turbidity, starch, dextran and colloidal silica. Approximately two thirds of the turbidity and colloidal silica were removed but only 20 percent of the starch and dextran.

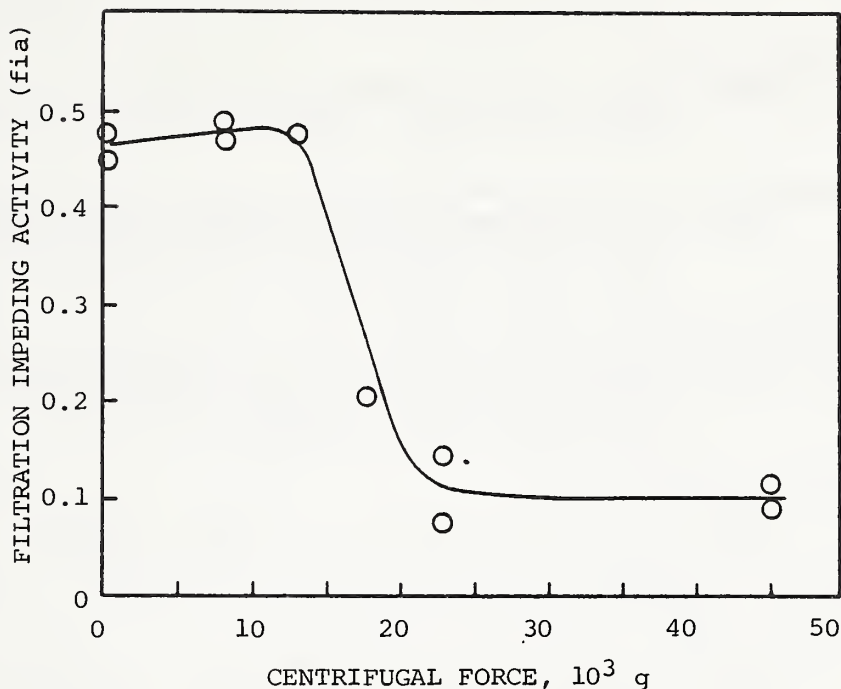


Figure 9.--Fia of the different impurity fractions separated from selected sugar samples

CONCLUSION

The individual effects of a large number of impurities on the filtration rates of liquors carbonated according to Australian industry practice have been quantified. It is not possible to state that all the important filtration impeding impurities were detected however for those impurities tested it is possible to determine the effect of their presence on carbonation refinery filtration rates.

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DISCUSSION

Michael J. Fowler, Amstar: I noticed that the second carbonatation was below pH 7.7. Is that a normal refinery pH?

McCowage: That pH is measured hot and is equivalent to pH 8.2-8.3 measured at 20⁰ C which is where we control our second carbonatation.

Fowler: In your paper, you indicate that soluble phosphate is probably the greatest interference to carbonatation filterability. Do you believe that the pH would have an effect on the phosphate interference with filterability?

McCowage: I can't comment on that. It's outside our area of experimentation.

Fowler: We notice that when we have poor filterability, sometimes raising the pH in the final carbonatation does help. In some cases we've observed that, but we hadn't considered phosphate as having an influence on filterability.

McCowage: Well, it's nothing we've looked at, and I can't think of any fundamental reasons as to why it might be better or worse. You are talking about precipitation of calcium phosphate, and as it is less soluble than calcium carbonate it is going to come down fairly liberally.

John C. Williams, Tate and Lyle: You mentioned at the beginning that you had the aim of predicting the filterability from an analysis of the raw sugar. How close do you think you are to being able to do that?

McCowage: We are a fair way from it because there are a number of tests we would have to run, and a number of the interfering compounds to be identify. The complex area is that of the polysaccharides. We would have to define threshold levels for the effects of starch, dextrans and gums. We don't have the techniques at present to be able routinely to fractionate polysaccharides into particular molecular weights. We determined in this study that the whole matter was fairly complex--as we thought it would be--but we thought it was a good time to start investigating the area as we do experience raw sugars that do not conform to predicted behavior by bomb filtration tests. Basically, we still use the Nicholson and Horseley approach to look at raw sugar filtering quality. Our knowledge is still not perfect, but now when we get a significant deviation, we can start looking for a number of known interfering impurities and we can give feedback to factories as to why they are having problems with particular sugars.

Margaret A. Clarke: I have several points to raise. First of all,

congratulations on excellent work. My first question is, would you explain the actual filter in the Nicholson-Horseley bomb.

McCowage: This is the kind of question where a picture would be worth a thousand words! The main apparatus is a brass filter which has a body approximately 50 mm in diameter and about 300 mm long. On the bottom of this is a retaining ring which holds a backing disc. We put a Whatman 54 filter paper over the backing disc, fill with the test solution, seal the top, and apply 50 psi pressure. The test solution is 60 Brix, buffered to pH 8.5, and we add 0.7% Celite 505 as a filter aid. Filtrate is collected between 2 and 7 minutes and the ratio between the amount of filtrate obtained in that 2-7 minute period for the test solution and a solution of pure sucrose expressed as a percentage is the filterability.

Robert Kunin, Consultant: At what pH did you run that test?

McCowage: pH 8.5

Kunin: You run the test at pH 8.5, but you do your carbonatation filtration at 7.5?

McCowage: The Nicholson and Horseley test doesn't reproduce carbonatation conditions. It is a test developed in the early 1950's as an indicator of raw sugar filtering quality.

Clarke: You did not use the Nicholson-Horseley filter in this current work?

McCowage: We didn't use that filter. In the carbonatation filtration studies we used a millipore membrane filter body which was jacketed and held at 80° C.

Kunin: And what was the porosity of the millipore?

McCowage: It was a Whatman No. 1 filter paper. We disregard the effect of the septum in this work.

Clarke: Do you add filter aid in the actual filtration after your carbonatation model?

McCowage: We don't add filter aid, just filter the carbonatated slurry. I might add, that's not how the factory filter station works. There is a precoat. Slurry resistance is determined simply by having a jacketed millipore filter body on the station. That is how we compare the small scale tests to the refinery.

Clarke: One other point I want to make: Although our work is not as detailed as yours, in many aspects, I'm happy to say it agrees with yours. One of the things we haven't looked at yet is phosphate. Dr. Chou looked at phosphate some years ago and he,

too, found that phosphate was responsible for a lot of hangups in filtration--directly after phosphatation, too, not just after carbonatation. I want to ask you if you want to speculate on the reasons for that--for the problems that phosphate ions give in filtration.

McCowage: In carbonatation, the calcium phosphate that is precipitated is very fine, whereas the calcium carbonate forms aggregates which are relatively large. The calcium phosphate blocks the interstices in the matrix formed by the calcium carbonate aggregates.

Clarke: That would apply in a carbonatation case but not necessarily in phosphatation. The question that Mike Fowler had about the difference in pH may apply here. There is a change in the calcium phosphate structure right around pH 8, and that may be the difference that you are observing there.

Richard Riffer, C & H: It changes from hydroxyapatite to calcium triphosphate.

Kunin: Magnesium phosphates, normally, are less soluble than calcium phosphates. So you have a real "witches brew" here with three components. The magnesium also reacts with the silicates so you have a four-component system which is pretty complex. When you add these impurities, is there an effect of time? In other words, when we start, all the components are present homogeneously and you get good precipitation when you carry it out. Now when you add these things to a sugar and carry it out, are the times related to letting these crystals grow or not grow a factor?

McCowage: I can't say. The residence time in the lab vessel at each stage is quite similar to the refinery. One thing I might expand on here is the work we did with starch. This was quite pleasing to us because it backed up some work that we'd done in Australia in the 1960's but were never able to satisfactorily explain. At that time our raw sugars had very high levels of starch and we were forced to use enzymes to get our filtration rates up. With enzyme addition, the reductions in measured starch were quite small, only about 50% reduction, and yet the filtrate volume per cycle might go up 5 or 10 times. The results discussed earlier explain why that happened. The enzyme was randomly attacking the starch molecule, chopping it up into smaller molecular weight sizes. While that starch was still sensitive to the starch-iodine test and was being measured it was not having an effect on filtration.

Clarke: How did you determine that the starch was all solubilized?

McCowage: We boiled the solutions for 20 minutes and tested them, and then we autoclaved them for 20 minutes, and we got no

difference in results.

Clarke: There can be different solubilities for the different types of starch that you mentioned. With regard to dextran, there is the contention (I suppose it has become that now) that the low molecular weight dextrans do not have much effect on refinery performance. This is the point of view that you have expressed with regard to the dextrans in your filter test in this study. Did you measure how much dextran came out of the carbonatation?

McCowage: No, we didn't.

Clarke: Do you know how much dextran of the various relative molecular weights was actually going onto the filter?

McCowage: Not out of the carbonation. In terms of the whole slurry, we knew how much we put in, but we did not know how much was in the filtrate and how much was entrapped in the calcium carbonate crystals.

Clarke: Yes, that's what I meant--the difference in what went into the carbonate crystal and what was actually getting onto the filter, impacting as a soluble polysaccharide. I think that might be important.

McCowage: I think this follows the trend we see with other compounds, the colorants, for instance--perhaps the higher molecular weight materials are being trapped and removed.

OBSERVATIONS ON FILTRATION IMPEDANCE IN RAW SUGAR

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INTRODUCTION

Studies on the effect of non-sugar components on filtration impedance and filterability problems of raw sugars are in progress. Experimental work has been conducted using a Walton filter, a single leaf model filter press. Initial results, on the nature of the factors to be studied and results at room temperature (25° C) were reported at the 1984 Sugar Industry Technologists' meetings (Devereux and Clarke 1984). The study examined the effect of soluble and insoluble solids in sugars on filtration, and was in agreement with literature reports that the level of total non-dissolved solids is a major determining factor in filtration impedance (Nicholson et al 1961, Hidi and McIntyre 1961, Yamane et al 1965, Tu 1968, Richards and Stewart 1974, Whayman and Meredith 1977). A Coulter counter determination of particle sizes showed that very small particles, of less than 4 μ diameter, had the strongest effect on filtration impedance. Electron microscopy indicated field soil particles made up part of this size fraction. Polysaccharides, especially dextran, including low molecular weight dextran, made a major contribution to filtration impedance by soluble solids.

Filtration impedance was read as the slope of a line plot of time over volume of filtrate (weight collected) versus volume of filtrate, as outlined in the following section on theory.

In the current study the filtration impedance of five washed raw sugars (WRS) of different origins was determined at temperatures of 70° C and 80° C; the data from 25° C (Devereux and Clarke 1984) are included in results in this paper. These sugars are described in Table 1 with regard to their filterability and general quality (color, pol, ash).

Table 1.--Filtration impedance of washed raw sugars and quality
(Poor impedance signifies poor filterability or high impedance.)

Sugar	Filt'n impedance		Quality
	25° C	70° C	
A	Poor	Very Poor	Low
B	Poor	Poor	High
C	Very Good	Very Good	Very High
F	Poor	Poor	High
G	----	Good	Low

Factors affecting filterability are considered as:

1. Viscosity
2. Suspended solids
3. Dissolved solids
4. Temperature

Sugars were analyzed for chemical components of both suspended solids and dissolved solids. Dissolved solids include polysaccharides, dextrans, amine compounds, phenolics and some inorganics. The effect of each type of component on viscosity was considered. Correlation coefficients for filterability with each type of component at the three different temperatures were calculated.

Electron microscope photographs of suspended solids of both acid-hydrolyzable and non-hydrolyzable (very insoluble) types, were obtained. Coulter Counter particle size distributions were obtained.

In the earlier paper (Devereux and Clarke 1984), starch was not considered, because the experiments were made at room temperature. For this study, at operating temperatures, all sugars were tested for their starch content. The physical nature of the starch, whether granular or gelatinized, was considered, in light of earlier observations that granules of starch remained in some raw sugars (Devereux and Clarke 1984, Parrish et al. 1985). Further work on starch forms in raw sugars at the authors' laboratory is described in another paper at this Conference (Parrish et al. 1985).

All tests were run on solutions of washed raw sugar at 50 Brix for room temperature runs and at 63 Brix for the 70° C and 80° C. All sugars were washed according to the standard Contract

#10 procedure (Devereux and Clarke 1984) so that the effect of over- or under- washing would not be a factor.

The type of filter aid is also not considered in detail in this paper. Standard Super-Cel was used throughout this study.

THEORY

Filtration can be analyzed as a fluid flow process, more specifically, a laminar flow through a packed bed. The driving force is the pressure drop across the filter cloth and the filter cake. The starting point to the analysis of the flow through the filter cake is given by McCabe and Smith (1976).

$$\frac{dP_c}{dL} = \frac{K_i \mu u (1-\epsilon)^2 (S_p/V_p)^2}{\eta_c \epsilon^3} \quad (1)$$

dP/dL = pressure gradient at thickness L
 μ = viscosity of filtrate
 u = linear velocity of filtrate base on filter area
 S_p = surface of single particle
 V_p = volume of single particle
 ϵ = porosity of cake
 K_i = constant
 η_c = Newton's law proportionality factor

u is given as

$$u = \frac{dV/dt}{A} \quad (2)$$

where V is volume of the filtrate collected in time t . As the filter area is constant, u is independent of L . The volume of solids in a differential layer dL is

$$A(1-\epsilon)dL \quad (3)$$

If ρ_p is the density of the particle, the mass of solids in dL is:

$$dm = C_p (1-\epsilon) A dL \quad (4)$$

Substitution back into equation 1 gives the pressure drop across dL as:

$$dP_c = \frac{K_i \mu u (S_p/V_p)^2 (1-\epsilon)}{\eta_c \rho_p A \epsilon^3} \quad (5)$$

If the filter cake is incompressible, i.e. made up of rigid

uniform particles, everything on the right of equation 4 is independent of L except M. Equation 4 can then be integrated over the whole filter cake to give:

$$\Delta P_c = \frac{K i \mu u (S_p/V_p)^2 (1-\epsilon)}{\epsilon^3 \rho} \quad (6)$$

where ΔP_c is the pressure drop across the entire filter cake.

The specific cake resistance is defined as:

$$\alpha = \frac{K i (S_p/V_p)^2 (1-\epsilon)}{\epsilon^3 \rho} \quad (7)$$

Substitution of equation 2 into equation 6 gives:

$$\Delta P_c = \frac{\mu \alpha u m_c}{g_c A} \quad (8)$$

Equation 8 is the pressure drop through an incompressible filter cake.

The filter medium is a resistance to flow and therefore contributed to the pressure drop. The filter medium is defined by McCabe and Smith (1976) as:

$$\frac{\Delta P_m}{R_m} = \frac{\mu u}{g_c} \quad (9)$$

P_m is the pressure drop across the filter medium.

The total pressure drop across the cake and filter cloth is:

$$\Delta P = \Delta P_c + \Delta P_m = \frac{\mu u}{g_c} \left(\frac{m_c \alpha}{A} + R_m \right) \quad (10)$$

The mass of solids collected can be determined by a material balance as:

$$m_c = V_c \quad (11)$$

where c is the mass of dry cake per volume of filtrate collected.

Substituting equation 2 and 11 into equation 10 gives:

$$\Delta P = \frac{\mu}{A g_c} \left(\frac{\alpha V_c}{A} + R_m \right) \frac{dV}{dt} \quad (12)$$

Under constant pressure drop, equation 12 can be integrated over time t and rearranged:

$$t/V / A = \frac{\mu}{\Delta P g_c} \left(\alpha c \left(\frac{V}{A} + R_m \right) \right) \quad (13)$$

To evaluate the constants K and R_m for a definite pressure drop, data of t vs. V should be taken. Equation 13 can be

written as the equation of a line:

$$t/V/A = \frac{K_i}{\Delta P} \left(\frac{V}{A} \right) + \frac{B}{\Delta P} \quad (14)$$

$$K_i = \mu \alpha C / g_c \quad (15)$$

$$B = R \mu / g_c \quad (16)$$

The cake resistance is dependent only upon the properties of the filter cake at a definite P. If the cake is incompressible, equation 13 will give a straight line graph.

Under conditions used in this study, the cake is considered incompressible, and readings are taken at constant pressure, after the initial precoat and build up with body feed.

Therefore equation 14 can be represented as:

$$y = ax + b$$

$$\begin{aligned} \text{where } y &= t/V \quad (V/A) \\ a &= (K_i / \Delta P) \\ x &= V/A \\ B &= B / \Delta P \end{aligned}$$

A, the surface area of the filter, is constant. Therefore, a plot of t/V vs. V will give a straight line with slope = $K_i/\Delta P$, the filtration impedance, in units of sq. in. - sec/ft. - cu. ft.

The slope, K_i/P is represented as F , filtration impedance. K_i contains the viscosity term, so $(K_i/\Delta P) (1/\mu)$ is represented by F/V , filtration impedance without viscosity effect. Since filtration impedance $K_i/\Delta P$ depends on (1) viscosity and (2) amount of solids in solution, both soluble and insoluble, F/V will be used to represent the effect of solids on filtration impedance with viscosity effect excluded.

MATERIALS AND METHODS

Filtration

At room temperature the procedure used was as follows: 1000 g of a 50 Brix solution of sugar was prepared. To 350 g of this solution was added 2.19 g Standard Super Cel and this was recycled as precoat for 8 minutes. Then 600 g solution, containing 0.75 g filter aid as body feed, was pumped through the filter. As the solution went through, pressure rose until at 25 psi pressure was maintained constant and filtrate collected for 13 minutes. This time was chosen as the optimum

for minimizing error and sample size.

The Walton filter cell is made of clear acrylic, total volume 6.67 cu. in. A wire mesh screen, area 3.14 sq. in. with a thin coating of silicone rubber on the diameter (a modification made by this laboratory) is the filter. Dexagauge pressure gauge, from 0 to 30 psi measured pressure, and flow rate is measured and controlled by a Masterflex controller and tachometer.

At elevated temperatures the procedure used was as follows: 1000 g of a 63 Brix solution of a washed raw sugar and 350 g of a 68 Brix solution of white sugar were prepared. These solutions were heated to the desired temperature. To the pure sugar solution, 1.0 g filter aid was added as precoat and the solution was circulated through a Walton filter-cell immersed in a constant temperature hot oil bath, until a pressure of 10 psi was reached in the filter. Then 1.5 g filter aid was added to the washed raw sugar solution and the solution was pumped through the filter at a high flow rate. As the solution went through, the pressure rose until at 27 psi pressure was maintained constant and filtrate collected for 10 minutes, and measured by weight on a top-loading balance.

The Walton filter cell used for high temperature testing differs from that used at room temperatures in that it is made of stainless steel and can be immersed in a hot oil bath for thermostatic control.

Analytical Procedures

Starch was analyzed by the S.M.R.I. method which includes boiling the sugar solution in 40% calcium chloride solution to achieve starch solubilization (Chen 1977). Starch granules were observed in samples of the centrifugate under polarized light on an Olympus Vanox Polarizing Microscope (Olympus Optical Co., Tokyo, Japan).

Viscosities were determined on an oscillation viscometer, Model 7.006 (Nametre Co., Edison, N.J.). The instrument was zeroed in air and calibrated in water. Densities were measured in 25 ml volumetric flasks.

Electron micrographs and x-ray dispersion element profiles were obtained on a Cambridge Stereoscan 250 scanning electron microscope with a Tracor Northern 2000 Energy Dispersive x-ray system.

Particle size distributions were run on a Model IAI. (Coulter Electronics, Inc., Hialeah, Florida) in aqueous suspension.

Surface elemental analysis by x-ray fluorescence was run on an

Ortec Energy Dispersive X-ray Fluorescence spectrometer, Model EEDSII. Sugars were run as 50 Brix solution, to eliminate crystal surface effects. Other elemental analyses were run by atomic absorption spectrophotometry at Galbraith Laboratories, (Knoxville, Tennessee).

Analyses of sugars in the acid-hydrolyzed polysaccharides were run by HPLC, using an HPX-87C column, with aqueous mobile phase, on a Sugar Analyzer I (Waters Associates, Milford, Mass.) Data were processed on a Data Module 730 (Waters Associates) which displayed a tracing of the chromatogram and calibrated the area against standards.

Dextran analyses were run by the Roberts Copper Method (Roberts 1982).

Total polysaccharides were run by the alcohol precipitate, phenol-sulfuric acid colorimetric method (Roberts 1980).

Phenolics were run by a colorimetric method using the Folin-Ciocalteu reagent (Godshall and Roberts 1982).

Total amino nitrogen was run by a ninhydrin colorimetric method developed by Smith (Smith 1971).

Separation of centrifugable solids was made by centrifuging 1000 g of 50 Bx solution for 20 min. at 27,000 G on an International refrigerated centrifuge, Model PR2. The centrifugate was then dialyzed on a rocking dialyzer against toluene saturated water for 4 days, to remove sugars and other solubles. It was found that washing the centrifugate in the centrifuge tube, and centrifuging under the same conditions three times, gave similar results to dialysis.

Hydrolysis of centrifugable solids was done by adding 50 mg solids to 50 ml of 2N H_2SO_4 and boiling under reflux for 4 hours. The solution was then filtered on a 0.45 μ Millipore filter. The non-hydrolyzables were washed well with water. The hydrolyzed filtrate was neutralized with barium hydroxide, and the excess barium precipitated with CO_2 from dry ice, and filtered off. This avoids excess salt formation in the hydrolysate.

OBSERVATIONS AND DISCUSSION

Dissolved Solids

Polysaccharides. When filtration impedance on the five sample sugars (four of these had shown filtration problems) was measured at operating temperatures, three of the four difficult to filter sugars showed either an increase in, or only a slight

drop in, filtration impedance from 70° C to 80° C as shown in Table 2. This is unlike the usual decrease in impedance at increased temperature, shown by sugar C. Upon observation under a polarized light microscope these three sugars all revealed ungelatinized starch granules in their centrifugate. Table 3 outlines these observations.

Table 2.--Filtration impedance at operating temperatures

Sugar	Filtration Impedance X 10 ⁵		
	25° C	70° C	80° C
A	7.46	64.6	86.9
B	8.11	6.5	6.5
C	2.77	1.7	1.4
F	4.95	3.6	3.7
G	-----	2.7	-----

Table 3.--Effect of starch granules on filtration impedance

Sugar	Granules observed	Impedance, 70° C	Change in impedance From 70° C to 80° C
A	Yes	High	Increase
B	Yes	High	Same
C	No	Low	Decrease
F	Yes	High	Increase
G	No	Medium Low	Decrease

The three sugars with ungelatinized starch granules were also the ones with the highest impedance. These very small granules were reported in sugar F in the earlier paper in this series (Devereux 1984). Further investigations into the nature and causes of these starch granules are reported by Parrish (1985).

Table 4 shows the correlation of filtration impedance with starch content at three different temperatures. The correlation was best at 80° C. The soluble starch effect was already evident at 70° C, and the impedance effect of the undissolved granules showed up more severely at the higher temperatures, lower viscosities and faster flow rates of 80° C conditions.

Table 4.--Effect of starch on filtration impedance

Sugar	Starch content ppm	Filt'n impedance x 10 ⁵		
		25° C	70° C	80° C
A	235	7.46	64.6	86.9
B	137	8.11	6.5	6.5
C	87	2.77	1.7	1.4
F	183	4.95	3.6	3.7
G	52	----	2.7	----
Correlation Coefficient, r, =		0.52	0.54	0.80

To test further the effect of the physical nature of starch, 400 ppm of starch, either treated, or non-treated, was added back to the good filtering sugar C. In one experiment 400 ppm of starch was added to deionized water and boiled for 20 minutes, then added to the sugar; the sugar-starch solution was then brought up to the filtering temperature, and the impedance determined. In the other case, 400 ppm of starch was added to water at room temperature. After stirring for 20 minutes with no heat, sugar was added, and the solution then brought up to filtering temperature and the impedance determined. Table 5 shows the effect of heat treatment of starch on impedance. The solutions with the unboiled starch had an extremely high filtration impedance; the starch granules were still intact in this solution. The solution which had the cooked, gelatinized starch showed only a modest increase in filtration impedance. No starch granules remained in this solution.

Table 5.-- Change in filtration impedance upon addition of 400 ppm starch

Sample	Filtration impedance x 10 ⁵	
	70° C	80° C
Sugar C, as is (87 ppm)	1.7	1.4
Sugar C, starch boiled & added	2.1	1.9
Sugar C, starch added (not boiled)	11.6	15.9

From Tables 1 and 3 it can be seen that both the content and the physical condition of the starch in a washed raw sugar are important factors in the filterability of washed raw sugars. The recent observation of ungelatinized starch granules (Whayman and Meredith 1977, Devereux and Clarke 1984) in raw sugars, some of which have been prepared with amylase treatment, sheds new light on filtration impedance.

Dextrans. There has been much discussion in the literature (Devereux and Clarke 1984, Nicholson et al. 1961, Hidi and McIntyre 1961, Yamane et al. 1965, Tu 1968, Richards and Stewart 1974, Whayman and Meredith 1977, Parrish et al. 1985, Murray 1972, Chou 1983) on the effect of dextrans on filtration impedance. There is agreement that dextrans decrease filterability but whether because of viscosity increase alone (unlikely (Richards and Stewart 1974, Whayman and Meredith 1977)), their contribution to total solids, or some other effect (Richards and Stewart 1974) is not known. It must be remembered that in these studies on actual sugars, dextran analysis was done by the haze method (Parrish et al 1985) which gives high molecular weight dextrans only. In this study, sugars were analyzed by the Roberts Method (Roberts 1982). Results with added known compounds show that low molecular weight dextrans, which are not assayed by the haze method, inhibit filterability.

Table 6 shows the correlation between filtration impedance and dextran content at different temperatures. An increase in temperature had little effect on the dextran filtration impedance factor. When viscosity was factored out, the correlation at 70° C was 0.69; including viscosity effect the correlation was 0.70.

Table 6.--Effect of dextran on filtration impedance

Sugar	Dextran Content ppm	Filtration Impedance x 10 ⁵		
		25° C	70° C	80° C
A	270	7.64	64.6	86.9
B	240	8.11	6.5	6.5
C	68	2.77	1.7	1.4
F	46	4.95	3.2	3.7
G	684	----	2.7	----
Correlation Coefficient, r, =		0.72	0.70	0.69

The effect of total polysaccharides is quite low at room temperature but increases significantly at elevated temperatures. "Total polysaccharides" includes dextran, starch, indigenous sugarcane polysaccharide, sarkaran, any dissolved bagasse polysaccharide or any other polysaccharide present. Table 7 shows the correlation between total polysaccharide content and filtration impedance. Viscosity difference caused by polysaccharide again plays a small role in filtration impedance. When viscosity is factored out of impedance, at 70° C, the remaining solids factor shows a correlation of 0.75, against 0.70 for the impedance.

Table 7.--Effect of total polysaccharides on filtration impedance

Sugar	Total polysaccharide ppm	Filtration impedance x 10 ⁵		
		25° C	70° C	80° C
A	1050	7.46	64.6	86.9
B	580	8.11	6.5	6.5
C	502	2.77	1.7	1.4
F	363	4.95	3.6	3.7
G	873	----	2.7	----
Correlation Coefficient, r, =		0.42	0.70	0.83

Low molecular weight dextrans. Dextran molecular weight and molecular structure are important factors in the physical effects of soluble dextrans. Result of addition of controlled amounts of low molecular weight dextrans to a good filtering WRS (sugar C) are shown in Table 8. To sugar C was added 1000 ppm of dextran T-10 (10,000 mol. wt.), and filtration impedance was measured. At each temperature the impedance increased when the dextran was added, as shown in Figure 1. Similar trials with Dextran T-40 showed impedance very close to that of T-10, and distinctly higher than that of the sugar when no dextran was added.

Table 8.--Effect of low molecular weight dextran on filtration impedance

Sample	Filtration impedance x 10 ⁵		
	25° C	70° C	80° C
Sugar C (68 ppm)	2.77	1.70	1.40
Sugar C + 1000 ppm T-10 dextran	3.19	2.00	----
Sugar C + 1000 ppm T-40 dextran	3.21	2.10	1.90

Earlier work done at room temperature (Devereux and Clarke 1984) showed that, although filtration impedance decreased as the dextran molecular weight decreases, there was still considerable impedance at low molecular weight.

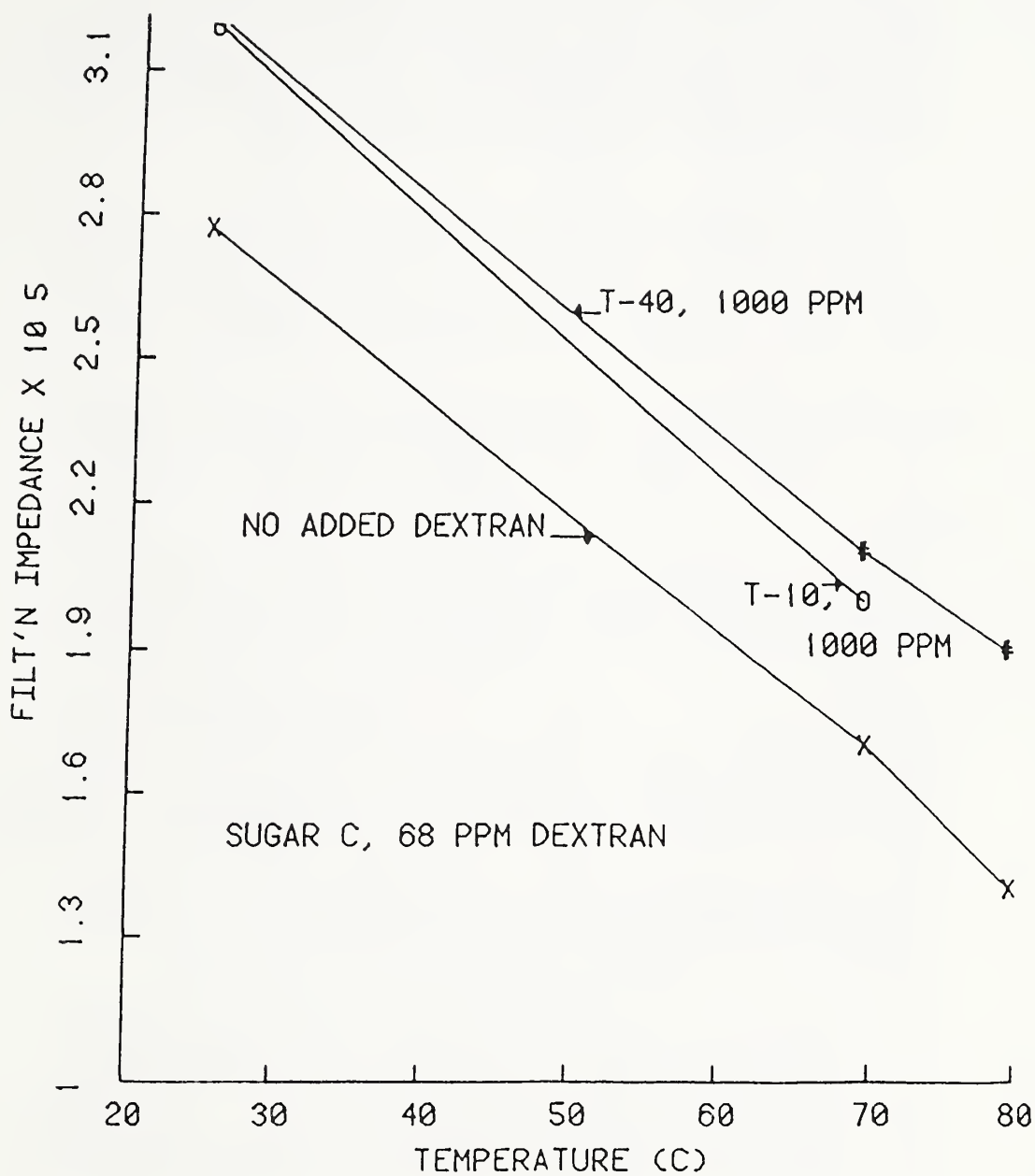


Figure 1.--Effect of low molecular weight dextrans on filtration impedance at increasing temperatures.

Amino Nitrogen. The amino nitrogen test indicates primary amines and therefore is a measure of the protein and amino acid content in sugars. It is expected that proteins, large molecules with many hydrophilic groups, should have an effect on both viscosity and water activity and therefore on filtration impedance. Table 9 shows the amino nitrogen content of the five WRS tested and the correlation with the filtration impedance. Correlation increased significantly with temperature, as was expected because of the tendency of protein compounds to pick up water and gelatinize at increasing temperatures.

Table 9.--Effect of amino nitrogen on filtration impedance

Sugar	Amino nitrogen content ppm	Filtration impedance x 10 ⁵		
		25° C	70° C	80° C
A	17	7.46	64.6	86.9
B	11	8.11	6.5	6.5
C	8	2.77	1.7	1.4
F	15	4.95	3.6	3.7
G	8	----	2.7	----
Correlation Coefficient, r, =		0.59	0.73	0.72

Suspended Solids. Suspended solids, as considered here, are the solids that could be centrifuged out under conditions described under "Methods and Materials". This suspended solids material is known to have severe filtration impedance. At room temperature suspended solids had by far the most severe effect on the filterability of WRS. At refinery temperature the effect of suspended solids content on impedance was also very marked. Table 10 shows the correlation of suspended solids content with filtration impedance at three different temperatures. At room temperature and at refinery temperatures the correlations were well above 0.9, supporting the thesis that by far the greatest contributing factor in filtration impedance of washed raw sugar is the total amount of suspended solids in the sugar liquor (Devereux and Clarke 1984). Sugar A, where the unusual effect of undissolved starch was predominant, was excluded (as an outlier) from the correlation calculations.

Table 10.--Effect of total undissolved solids on filtration impedance

Sugar	Solids content (centrifugable)	Filt'n impedance x 10 ⁵		
		25° C	70° C	80° C
A	2.84	7.64	64.6	86.9
B	4.27	8.11	6.50	6.50
C	1.5	2.77	1.70	1.40
D	2.6	4.95	3.20	3.70
G	2.0	----	2.7	----
Correlation Coefficient, r, =		0.97	0.23	0.02
r, excluding "A", =		0.97	0.97	0.99

Several approaches taken to determine the nature of this solid material are described in detail in the authors' paper on room temperature filtration (Devereux and Clarke 1984).

Table 11 summarizes the correlation coefficients between each non-sucrose factor and the filtration impedance, for each temperature. Figures 2 and 3 show graphically the change in effect of each non-sucrose factor with temperature. The data in Table 11 emphasize the effect of total solids at all temperatures, and the contribution of total polysaccharides to the total solids effect.

Table 11.--Correlation of filtration impedance with factors studied

Factor	Correlation with filtration impedance		
	25° C	70° C	80° C
Total solids	0.97	0.97	0.99
Total polys'd	0.42	0.70	0.83
Starch	0.52	0.54	0.80
Dextran	0.72	0.70	0.69
Amino nitrogen	0.59	0.73	0.72

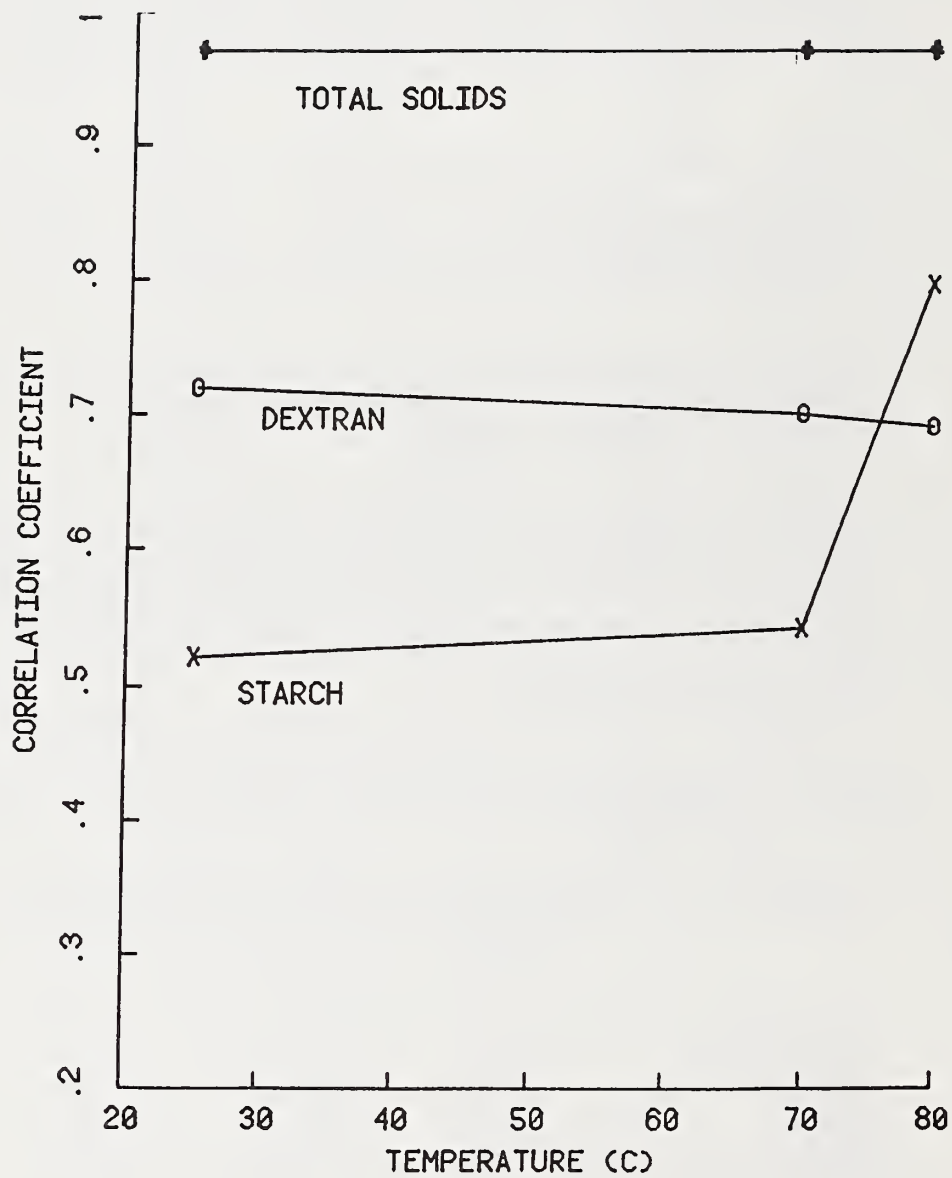


Figure 2.--Correlation coefficients of filtration impedance factors at increasing temperatures.

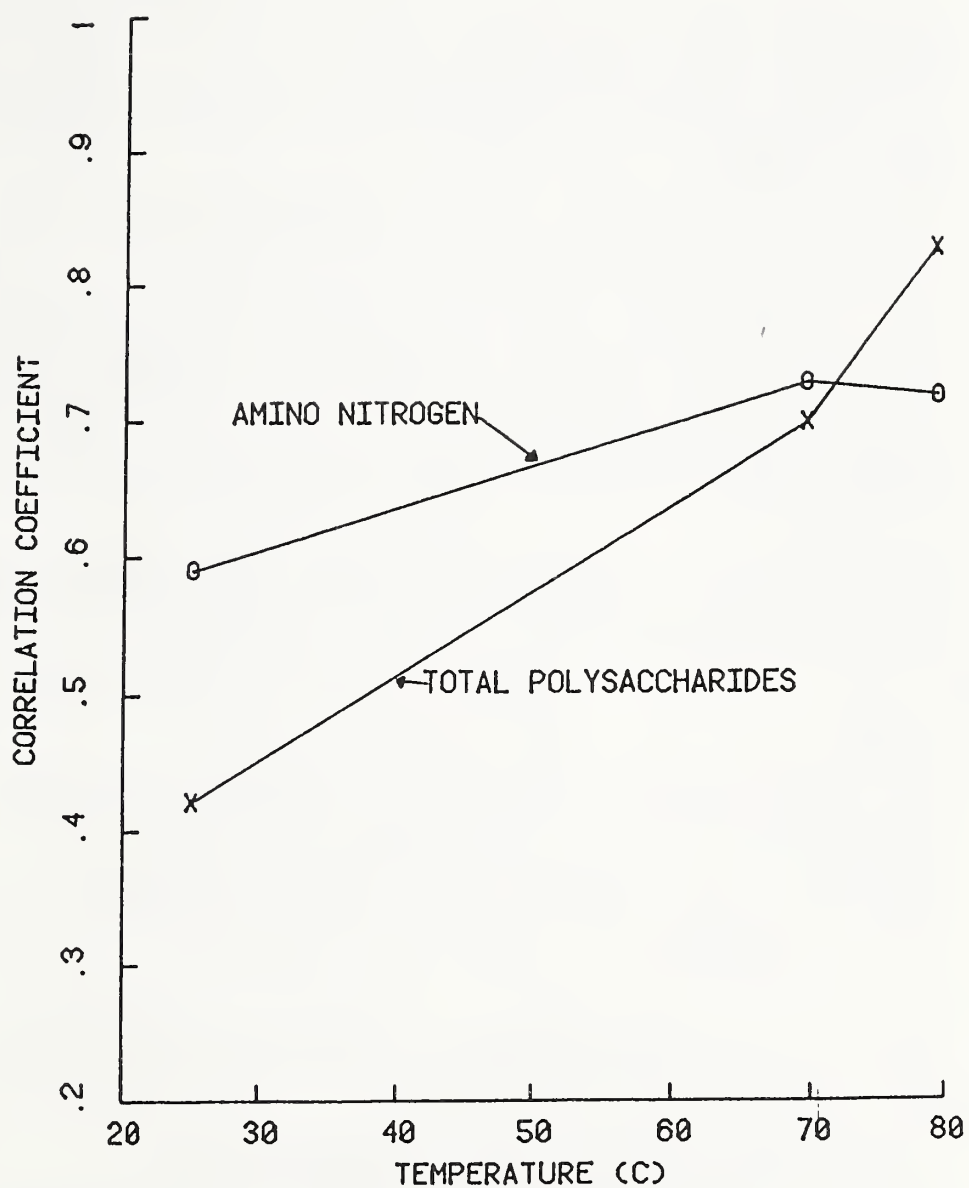


Figure 3.--Correlation coefficients of filtration impedance factors at increasing temperatures.

Table 12.--Correlation coefficients for filtration impedance and solids factor

Factor	r, Filtr'n impedance at 70° C	r, Solids factor f/v, at 70° C
Suspended solids	0.97	0.95
Starch	0.54	0.74
Dextran	0.70	0.69
Total polysaccharide	0.70	0.75
Amino nitrogen	0.73	0.72

Correlation of impedance with viscosity: $r = 0.34$

In light of the importance of solids to the impedance, the "solids factor" contribution to filtration impedance (impedance without viscosity effect) is charted in Table 12. Total polysaccharides and starch are major contributors to the solids effect. Amino nitrogen also shows a surprisingly high correlation.

The correlation of suspended solids with filterability is high, as expected, and suggests a possible "quick and dirty" test for filtration efficiency.

SUMMARY

In summary, the effects of soluble and insoluble non-sucrose components of washed raw sugars upon filtration have been studied at process operation temperatures. A filtration impedance factor has been devised, and correlated with properties of the sugars studied, and properties of some non-sucrose components.

The suspended solids content is confirmed as the strongest indicator of filtration impedance at operating temperatures, as well as at room temperature.

In the soluble solids fraction, the effect of total polysaccharides increases as temperature increases. Dextran remains the most significant polysaccharide to affect filtration, with consistent correlation across the temperature range. Low molecular weight dextrans contribute to filtration impedance.

Two effects are observed from starch in raw sugar: The well-

known decrease in filtration efficiency as soluble starch gelatinizes, and a new effect of insoluble starch contributed to the insoluble solids fraction. A new type of insoluble starch granule, of diameter under 2 μ , has been observed in some difficult filtering sugars. The effect of this non-gelatinized starch is discussed.

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DISCUSSION

Stephen A. Brooks, Barbados Sugar Research Institute: In the measurement of the total polysaccharide, did you include the fine granule type of starch?

Devereux: Yes, the measurement of total polysaccharide includes the fine granules.

Mark Wnukowski, Amstar: I was curious to know whether or not you did any experiments where you took two of your components and determined if the filtration impedance is additive. For instance, if you added starch and then spiked it with dextran, would you get an additive effect?

Devereux: No, we have not added two impurities at the same time to the washed sugars. We have not yet looked for an additive effect.

Robert J. McCowage, CSR, Ltd.: I have a few points. The first one, I think might be useful to you in doing impurity addition work. The effect of an impurity addition can depend very much on the other filtration impurities present. If you add a particular quantity of impurity to a very good filtering sugar, you're on a very steep part of that particular relationship so the relative effect will be large. If you take an average sugar which is further down the curve or a poor filtering sugar, the marginal increase of that additive effect is going to be much smaller. By putting it on a logarithmic relationship, you could get it on an equal basis.

Devereux: Yes, you're right. In some earlier work, we added dextran to sugar F, which was a very poor filtering sugar, and also to sugar C, a good filtering sugar. The filtration impedance of sugar F was affected less than that of sugar C. These results are not included in this paper.

McCowage: I think something you have to be a bit cautious about is talking about differences between impurities when working with correlation coefficients of around 0.6 to 0.7, with 5 or 6 data points. I think drawing conclusions from that number of data points with that degree of correlation becomes a bit dubious.

Devereux: That is a good point. We are not, however, stating quantitative effects for a refinery, i.e. for each amount of dextran in sugar, the filtration impedance is increased by so much. We are trying to show the relative effects of various components.

McCowage: Thanks. One more point. I wonder if you might explain to me the mechanism of the effect of dextran on water activity and, in the light of that, did you measure water activity? That's a fairly simple procedure to undertake.

Clarke: No, we didn't measure water activity. Any dissolved solids are going to change the water activity coefficient, first to lower it and then, at higher concentrations, to raise it. That is a very complex question. Perhaps you can give us some information on it.

Leif Ramm-Schmidt, Finnish Sugar: In practice, when we have a difficult sugar that we are running normally at 80° C, throughput is worse at 70° C because, although there are many things that increase the impedance when we go up in temperature, going down in temperature again, we have an increase in viscosity. Do you think, let's say on average, it's worth trying at a higher temperature?

Devereux: At two different temperatures, 10° C apart, there is a significant difference in the behavior of starch granules. Starch is the impurity that is most affected by the temperature rise, and the effect of the temperature rise is to decrease filtration rate.

Ramm-Schmidt: You mean, if we had high starch we would perhaps get higher throughput in the plant if we go down in temperature?

Devereux: That can depend on the nature of the starch. If the starch is solubilized, and the temperature raised, the filtration impedance will decrease. When we added nongelatinized starch and raised the temperature, the filtration impedance increased. In general, the starch in raw sugars is not gelatinized, but becomes so as the temperature is raised, increasing viscosity severely between 70° and 80° C.

Andrew Ho, Redpath Sugars: I'd like to ask a few questions. The first one, did you have any enzyme-treated raw sugar? Did this somehow change the natural starch?

Devereux: The sugars in which we found the greatest quantity of small starch granules had been treated with amylase. Apparently the amylase did not affect the granules. To check that amylase was not creating the small granules, we added amylase ourselves to sugars and found no formation of small granules. The addition of further amylase did not affect these granules.

Ho: Did you find any differences between using the various types of starch, such as starch of corn origin or other origin?

Devereux: We used only corn starch to add back to sugars.

THE CHEMISTRY OF IRON IN THE SUGAR REFINERY

Richard Riffer

California and Hawaiian Sugar Company

ABSTRACT

Iron contamination in soft sugar can adversely affect product quality and color stability. Removal may be a formidable task because of the high affinity of iron for other non-sugars. Moreover, iron-free liquors can readily pick up new iron. The roles of phosphate, pH, and light in autoxidative darkening during storage are addressed, as well as our progress in iron removal.

INTRODUCTION

The status of iron in the sugar refinery is unique on several grounds. First, iron may be inadvertently added during processing through contact with metal surfaces. In addition, iron is more difficult to remove than certain other common cations. Resins that readily remove calcium, for example, may do considerably poorer with iron. Char treatment may result in an increase in iron level, because liquors readily pick up iron in the char itself. Finally, iron is singularly troublesome in its impairment of color stability in soft sugars.

Although iron is an essential nutrient in our diets, its level in soft sugars tends to be in a kind of twilight zone--too low to prevent anemia in consumers but high enough to cause headaches in refiners.

Our laboratory studies have addressed these problems and, we believe, have helped to clarify the role of iron in the refinery. We have also made some progress in the difficult task of iron removal from soft sugars.

CONSEQUENCES OF IRON CONTAMINATION

Iron is known to accelerate darkening in soft liquors and sugars, acting as an oxygen carrier for autoxidation. Iron

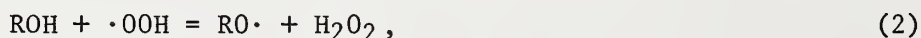
compounds have a high degree of catalytic activity, especially for promoting oxidations, their principal function in animal metabolism.

There are features of soft sugar darkening suggesting that phenols are involved. First, the photochemical/free radical contribution: in accelerated darkening-in-storage studies in soft sugars at moderate temperatures (55°C), we found that color formation was sharply inhibited by excluding light. Second, the gradual reddening of samples during storage, even at room temperature, is suggestive of quinone and phenoquinone formation. Another light-sensitive non-sugar, 5-hydroxymethyl-2-furaldehyde (HMF), turns yellow, not red. Further, the observation that addition of iron results in an immediate green-black pigmentation indicates the presence of catechol groups.

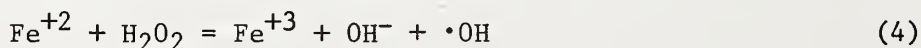
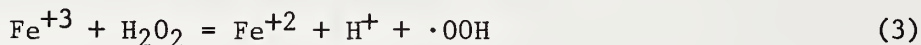
Since granulated sugar darkens very little in storage, the implication is that non-sugar components are serving as chromophore precursors and possibly photosensitizers as well. In the dark, iron itself can function as a free-radical source:



Following H-abstraction



both oxidation states can be regenerated:



We demonstrated the effect of iron to be purely catalytic. An iron-free low purity liquor was prepared using a chelating resin. This sample darkened much more slowly at 75°C than an untreated sample, but after six hours the iron-free sample "caught up" in color. This suggested that (1) the effect of iron is solely on reaction rate, and (2) new chromophore precursors are either not being generated at all, or else at a rate very slow compared to that of the darkening reaction itself. We also demonstrated that in an inert atmosphere, iron is without effect.

Oxidation also results in colorless products such as lactic, formic, and glycolic acids. Invert can be oxidized by such mild agents as Cu^{+2} ($E^\circ = 0.167$), and the reaction with Fe^{+3} ($E^\circ = 0.771$) would have an even more favorable enthalpy. Kinetics studies have shown that glucose is more sensitive to oxidation by iron than are other hexoses (Krupenskii 1983).

Apart from its oxidative properties, iron can affect soft sugar appearance and flavor, and liquor viscosity. Greenish soft sugars result from high levels of complexed iron. Indigenous

sugarcane polysaccharide, even at 1000 ppm, does not increase liquor viscosity except in the presence of ferric ion, which apparently crosslinks the chains (Sugar Processing Research, Inc. 1983). At high levels, iron adversely affects flavor, introducing perceptible metallic and astringent notes at 5-10 ppm in 20% solution (Sugar Processing Research, Inc. 1984). High concentrations also interfere with crystallization (Hernández León 1984).

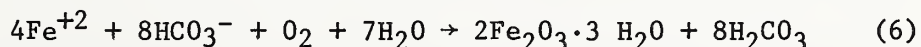
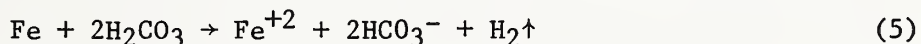
SOURCES OF IRON CONTAMINATION

In moist air iron is rapidly oxidized to a hydrous oxide, which affords no protection because it flakes off, exposing fresh surface. It also dissolves readily in dilute acids. Low pH liquors and sweetwaters, resulting from chemical or microbiological degradation, rapidly leach iron oxides from unlined surfaces of char filters, tanks, and pipes. The oxides can also be solubilized by complexing groups in non-sugars, irrespective of pH (Blesa *et al.* 1984). In addition, the oxides can be worn from such surfaces by abrasion, particularly in the char regeneration cycle.

A portion of the oxide is magnetic and can be removed from char using magnetic separators. Iron scale forms three layers of which the middle is the ferromagnetic Fe_3O_4 . Its magnetic property is a manifestation of its unique crystal structure, which is of course lost upon solution. Thus dissolved iron cannot be removed from a liquor magnetically. Fe_3O_4 is ferromagnetic up to its Curie temperature of 768°C ., where it becomes paramagnetic.

There is some evidence that oxidative loss of carbon from char during kilning is accompanied by oxidation of iron--presumably FeO , the form most soluble in acid--to ferromagnetic Fe_3O_4 . Although only minute amounts of iron oxides are adsorbed onto the char, a large fraction of the char may thereby display magnetic properties.

Iron rust originates commonly from the carbonate, which is subsequently oxidized to paramagnetic Fe_2O_3 . Initiation is typically by carbonic acid, but other acids behave similarly.



The first step does not take place readily with pure iron because of the over-voltage effect of hydrogen on the surface, but impurities facilitate escape of the gas. Note that a single oxygen molecule oxidizes four atoms of ferrous iron.

We found that a soft liquor sample, rendered iron-free using a chelating resin, rapidly picked up iron from char. This appears to be due to the high affinity of complexing groups in

the non-sugars for iron. Hence iron tends to be concentrated in the soft sugar system, where it is difficult to remove.

INORGANIC COMPLEXES OF IRON

Iron removal in the manufacture of granulated sugar does not ordinarily pose a serious challenge. Raws typically contain about 5-20 ppm, and levels can be reduced to well under 1 ppm as a result of processing. Iron removal, however, does not follow the course of alkaline earth removal on bone char. Instead its elimination is largely linked to removal of its complexes with non-sugars by affination and clarification, and to a lesser degree by crystallization. A small portion of the iron may be present as ferric hydroxide, removable by filtration.

Because of the unusually small solubility product of its hydroxide, 1.1×10^{-36} , ferric ion cannot exist in concentrations greater than 0.01 ppm in the pH range 5-8. Even at pH 2-3, the extent of hydrolysis is very high, and to have 99% of the violet $\text{Fe}(\text{H}_2\text{O})_6^{+3}$ ion, the pH must be near zero. On the other hand, appreciable levels of ferrous ion can be present, as a result of its relatively much larger constant, 1.64×10^{-14} . At pH 7 it is therefore possible to have ferrous ion present at concentrations up to 1.64M. Consequently uncomplexed ferrous iron can be removed by char or cation resin, if care is taken to prevent oxidation. Granular carbon, not surprisingly, is not especially effective.

Other species may be present, such as FeCl^{+2} , depending on pH and anionic environment. Phosphate forms such as $\text{Fe}(\text{HPO}_4)^+$, $\text{Fe}(\text{HPO}_4)_3^{-3}$, and $\text{Fe}(\text{PO}_4)_3^{-6}$ can be important. Such species will be dealt with in a separate section. Both ferrous and ferric ion most commonly have a coordination number of six, with identical d^2sp^3 hybrid orbitals oriented toward the vertices of an octahedron. The complement of six may be satisfied partly or wholly by water molecules.

Donor groups tend to be basic, and therefore hydrogen ions are in competition with iron for the groups. Consequently a decrease in pH shifts metal complexes toward dissociation, and all would decompose completely if the liquor were made sufficiently acidic.

ORGANIC COMPLEXES OF IRON

The relative concentrations of the various complexes of iron, both organic and inorganic, will depend upon the pH, the concentrations of donor groups present, and the relative stabilities of their complexes. There is competition with the donor groups or ligands by hydroxide, especially at high pH. The direction of the reaction depends upon the stability of the complex relative to the insolubility of the hydroxide. Another way of looking at the relationship is that non-sugars acting as sequestering agents may prevent precipitation of $\text{Fe}(\text{OH})_3$ up to high pH.

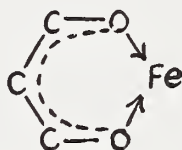
only must the complexes present be quite stable, but there must also be present a relatively high concentration of complexing species.

Assume that the concentration of potential ligands in the non-sugars is roughly equal to that of iron, or $7 \times 10^{-5}M$. If all of the free iron has originated from complex dissociation and most of the iron is not present as the hydroxide, then

$$[Fe^{+3}] < 10^{-15}, \text{ and } K_{dissoc.} = \frac{(<10^{-15})^2}{7 \times 10^{-5}} = <1.4 \times 10^{-26}.$$

Although the reasoning here is admittedly on the circular side, it does suggest a highly stable complex. We suspect that the concentration of potential ligands is actually higher than 7×10^{-5} , which would result in an even smaller dissociation constant.

We dialyzed a sample of sugarcane colorant (cutoff 3500 daltons) for elemental analysis and found an empirical formula of $C_{18}H_{24}O_{15}N$. The high level of unsaturation in a highly oxygenated material strongly suggests the presence of phenols and/or enols. Such groups have a much higher affinity for iron than does carbonyl or even carboxyl, and we believe these groups play an important role in sequestering iron. Other contributors could be ligands with lower affinity but a spatial arrangement that permits chelation, such as 1,3-diketones, with tautomeric enol forms:



THE EFFECT OF PHOSPHATE

Phosphate plays a crucial role in iron function. It acts as an antioxidant by complexing iron, but in addition appears to convert iron complexes to polyvalent forms more readily removed by adsorbents. This will be discussed in another section. Phosphate is not, however, without its drawbacks. Low levels are desirable in liquors going onto char, and perhaps also onto anionic resin, because polyvalent anions inhibit decolorization.

Interpretation of studies using added phosphate is complicated by the fact that oxidative darkening appears to be subject to a secondary salt effect. This is because low pH color formation is subject to general acid catalysis, and the high pH reaction to general base catalysis: the activities of weak electrolytes vary with the ionic strength of the medium. As a result, buffers of all types tend to participate in the darkening.

The level at which added phosphate significantly improves the color and stability of soft liquor is, very roughly, $5 \times 10^{-3}M$. The phosphate complex with iron appears to be less stable than that with certain other non-sugars, but at sufficiently high concentration a beneficial effect is achieved.

There is evidence that the effect of phosphate is only on reaction rate. Darkening is slowed by added phosphate but not prevented, as shown by these experiments with soft liquor saturated with air at 80°C.:

<u>color units generated</u>		
	<u>450 ppm added phosphate</u>	<u>no added phosphate</u>
2 hours	0	602
4	683	1360
6	2720	2850

This is consistent with our results demonstrating the effect of iron to be purely catalytic.

We further established that oxidation--but not caramelization--could be prevented by the use of an antioxidant:

<u>color units generated (80°C)</u>		
	<u>sat'd with N₂, no additives</u>	<u>sat'd with air, 100 ppm hydroquinone</u>
4 hours	1480	1530
6	2010	2060

COLOR STABILITY IN SOFT SUGAR

Soft sugar components which may be considered suspect in darkening are: (1) high invert levels, because of the ease of oxidation of aldehydes by air; (2) iron, because of its catalytic activity; (3) phenolics, because of their ease of autoxidation; (4) colorants, which could act as sensitizers in free radical formation; (5) weak acids, which could catalyze oxidation, but some of which could sequester iron; and (6) ash, which could have a minor catalytic effect. Although moisture is present, to allow for some diffusion of reactants, soft sugar is a sort of glassy intermediate between solid and solution.

We conducted kinetics studies on darkening reactions in soft liquor, using samples that were heated at constant temperature either in air or in an inert atmosphere. The data suggested that a fast oxidative darkening was occurring concurrently with a slower thermal caramelization. The latter reaction was found to display first-order kinetics, with an activation energy of

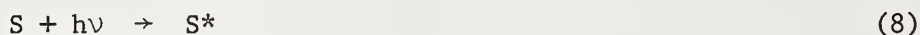
about 3.8×10^4 joules/mole, corresponding to a rate increase by a factor of about 1.6 for every 10°C . temperature rise.

The darkening reaction of soft sugar during storage displayed approximately zero order kinetics, ($E_a=9.08 \times 10^4$ joules/mole; but this is undoubtedly a catalytic-iron "reduced" value) indicating a surface reaction. It also suggested that air is strongly adsorbed on the crystal surface. Oxidation of invert by free radical chains could exhibit complex kinetics, but the reaction products might not be those being measured when the rate is followed by color formation.

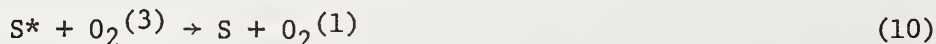
The darkening is not diffusion-controlled because it displays an appreciable temperature dependence. Diffusion has a \sqrt{T} dependence and chemical reaction an $e^{-E/RT}$ dependence. The darkening is accompanied by a pH drop. Since pH sensitivity is characteristic of many colorants, this could be responsible for apparent departures from zero-order kinetics after long storage.

Reflectance color increases are offset initially as more grain is formed in the syrup phase as the sugar cools. A gradual increase in moisture content during storage would melt small grain most subject to surface oxidation, again offsetting reflectance darkening.

Certain soft sugar colorants could act as sensitizers which absorb light and subsequently react with a phenol in a homolytic dehydrogenation to give the phenoxy radical (Grossweiner and Zwicker 1961):



S^* can also react with molecular oxygen to give (activated) singlet oxygen (Foote 1968):



However there are no published data that suggest that singlet oxygen is involved in the photochemical autoxidation of phenols. Enols, perhaps present in the non-sugar fraction nominally as β -diketones, are also subject to photosensitized autoxidation and photoinduced rearrangement (Becker 1971). Such substances also form complexes with transition metals that readily undergo oxidation and polymerization (Belcher *et al.* 1973). Disaccharides themselves are also known to produce stable free radicals by ultraviolet irradiation (Kashiwagi and Enomoto 1981).

POSSIBLE ROLE OF PHENOLICS

As mentioned earlier, the evidence for phenolic involvement in soft sugar darkening is circumstantial. However catechols are known to be present in non-sugars, and although they represent

a small fraction of the total, they could be important because of their high affinity for iron and their susceptibility to free-radical oxidation. Catechol itself is oxidized to 2,5-dihydroxy-1,4-benzoquinone (Ettel and Pospíšil 1957) by atmospheric oxygen, which perhaps behaves as a free biradical. Unless phenols are involved, it is difficult to explain how a resonance pathlength can be increased via autoxidation.

The oxidation kinetics of catechol in solution obeys the following rate law in the pH range 6.5 to 8.5 (Joslyn 1935):

$$v = k_1 \frac{[\text{cat}^-] [\text{O}_2] [\text{cat}]}{k_2 + [\text{cat}]} \quad (11)$$

Cat⁻, cat, and O₂ function in chain initiation and propagation. The appearance of cat in the denominator suggests that the species also functions in chain breaking. Any chain-breaking function of the solvent would affect the magnitude of k₂. Although this rate law does not conform to our observations in "solid state" soft sugar, it should be borne in mind that the latter darkening does not occur so rapidly as to suggest the formation of chains.

Joslyn observed a higher activation energy for the reaction in the presence of phosphate buffer. He attributed this to inhibition of catechol ionization, but it is conceivable that complexation of catalytic amounts of iron was responsible at least in part.

One-electron-transfer oxidants such as Fe⁺³ oxidize 4-methoxyphenols to quinones; oxidative coupling also occurs (Hewgill and Hewitt 1967; Müller *et al.* 1964; Posternak *et al.* 1948), leading to complex condensation products. Such reactions typically exhibit pronounced acceleration by transition metals (Fe, Cu, Mn) as well as by increased pH.

Photolysis of phenols at low temperature results in the homolytic cleavage of the O-H bond to give phenoxy radicals in low yield (Land *et al.* 1963):



Photochemical intensification of color could also occur by hydroxylation of phenols via reaction with OH[•]. Phenol in the presence of peroxides forms catecholic products in 34% yield (Omura and Matsuura 1968; Joschek and Miller 1966); such substances form highly colored iron adducts. Substituted phenols behave similarly.

Catechols hydrogen-bond to proteins, with a particularly high affinity for collagen, because of its open configuration and unusually high content of glycine, proline, and hydroxyproline. (Hydrophobic micro domains in the protein interior may also participate in the interaction.) Oxidation disrupts hydrogen

bonding by converting donor hydroxyls into acceptor quinone carbonyls, which can, however, form covalent linkages to lysine amino groups or cysteine sulfhydryls.

We made use of this "tanning" reaction, using purified hide powder, to study the fate of catechols in soft sugar during storage. The level of total phenols was also followed, using the highly sensitive Prussian blue method (R. Budini *et al.* 1980). Catechol was used as a standard (molar absorptivity 1.82×10^4).

We found that in the presence of strong ultraviolet light, the level of phenols rapidly rises, presumably by hydroxylation of the resonance-stabilized phenoxy radical. Upon long-term storage in the dark, both catechol and total phenols levels drop, suggesting oxidation to quinones. An alternative explanation for the observed decline in tanning activity, molecular weight decrease below 350 daltons, runs counter to the well-known acid-catalyzed polymerization of such substances. Moreover, this would not account for the concomitant decrease in total phenols.

Phenols actually inhibit free radical oxidation but are themselves oxidized in the process. They are so effective at trapping radicals that they are frequently used as scavengers. The point is a confusing one, but rate laws such as equation (11) offer clarification. Species in the numerator function in chain initiation and propagation; those in the denominator, in chain breaking. The relative magnitudes provide information on the degree of inhibition.

COLOR DEVELOPMENT STUDIES USING ADDITIVES

Synthetic soft sugar samples were prepared by coating a very fine granulated fraction with a soft sugar syrup in a mechanical mixer. The syrup was first treated with an additive, chosen to provide specific information on the color development reactions. Accelerated darkening was achieved by storing the samples at 55°C. The properties of the reaction are such that one day of such storage was equivalent to 28 days at 30°C., or 55 days at 25°C. Daily reflectance and solution colors, as well as pH, were monitored. The following additives were tested:

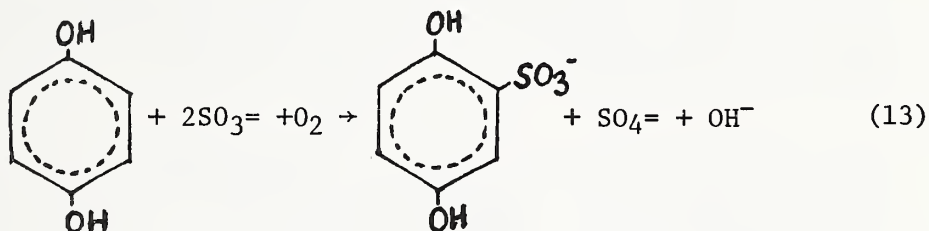
Iron - Added ferric ion sharply accelerated color development.

Phosphoric Acid - Darkening was strongly inhibited, but not merely from a pH effect, because acids with comparable pK_a values, such as bisulfate, were less effective. This could be assessed only approximately, because the relation between ionization constant and catalytic potency is logarithmic, not linear. The statistical factor for tribasic H_3PO_4 was ignored on the grounds that $H_2PO_4^-$ and HPO_4^{2-} are extremely weak compared to H_3PO_4 . Treated samples were also less green, probably due to disruption of complexes with iron, for which phosphate has a high affinity.

Citric Acid - This additive, which also complexes iron, inhibited darkening in excess of its pH effect.

Ascorbic Acid - This antioxidant, surprisingly, did not retard darkening. However ascorbic acid is known to be ineffective against iron, but effective against copper.

Bisulfite - This was an excellent inhibitor of color development, again implicating free radicals and phenolics: bisulfite is used as a stabilizer for hydroquinone-type photographic developers:



Quinone is formed in the absence of sulfite.

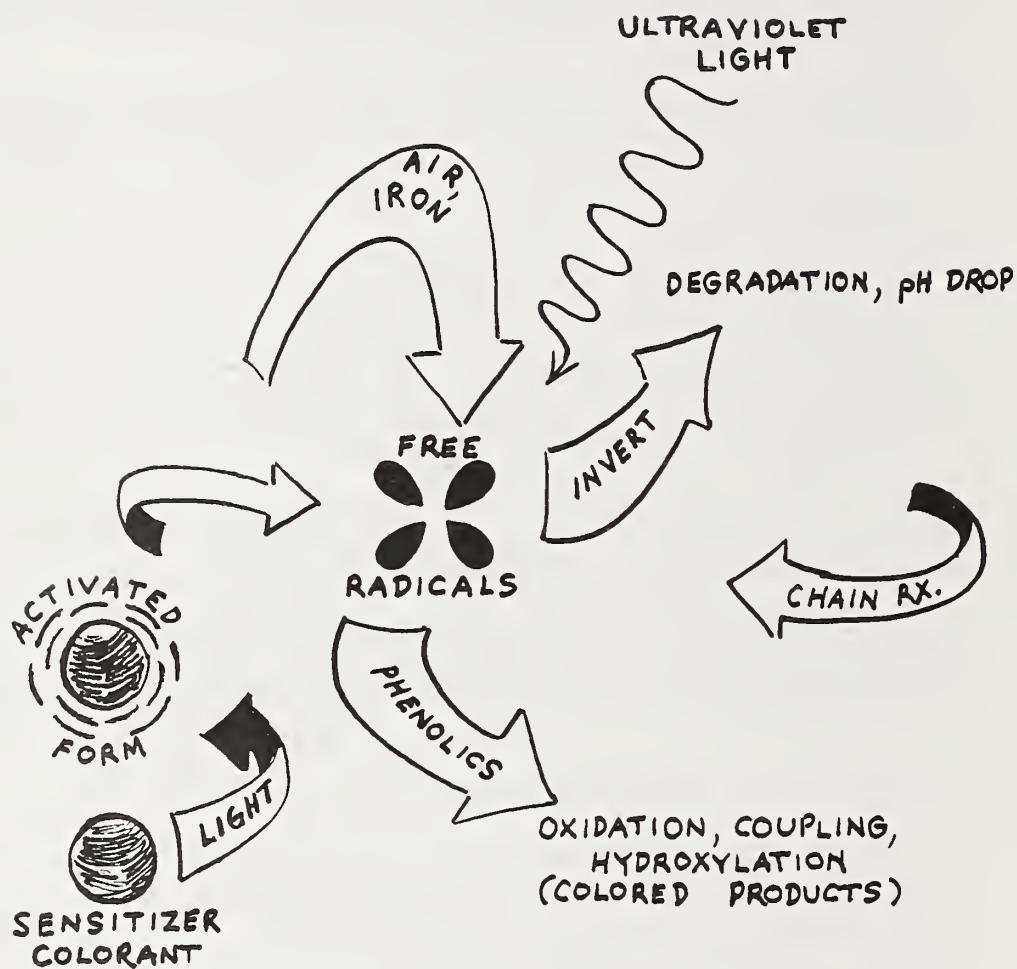
Hydrosulfite - This rapidly reduces ferric iron but did not retard darkening. The implication is that iron involvement is catalytic only; that is, ferric ion concentrations are too low for it to act as a principal (direct) oxidant.

INHIBITION OF DARKENING BY PHOSPHATE

How does complex formation reduce the redox potential? The potential is determined by the ratio of concentrations of Fe^{+3} and Fe^{+2} in equilibrium with the complex ions (Grinberg 1962). The normal couple is 750 mV; that for the ferrocyanide couple is 420 mV; and the oxalate (pH 7), 20 mV. The pyrophosphate couple near pH 7 is -110 to -175 mV, that is, it is actually reducing compared to hydrogen.

Since ferric ion is more acidic than ferrous, it would have a higher affinity for a given ligand. The presence of complexing groups should therefore increase the tendency of iron to be oxidized to the higher valence state, that is make it a better reducing agent. Other contributing factors are the geometry of the complex ion, the degree of covalent bonding, and the extent of solvation.

It is clear that complexed iron can have a reduced redox potential. Less obvious is the role of complexed iron functioning as a free radical source. However the combination of a ligand with Fe^{+3} could result in electron transfer to the iron, with the formation of a free radical in the ligand.



POSSIBLE ROUTES TO DARKENING IN SOFT SUGAR

STRATEGIES FOR IRON REMOVAL

In the manufacture of granulated sugar, iron removal is simply non-sugar removal. However circumstances are quite different in the low purity system. Here non-sugars are an essential component of the product, and we are faced with the problem of removing iron from complexes that may be quite stable.

The only refinery treatment specific for removal of iron of which we are aware is British Columbia Sugar's synthetic sulfonated tannin resin (Blankenbach 1953). Tannin is rich in phenolic groups, which are known to bind trivalent iron strongly; thus tea interferes with absorption of dietary iron. B. C. Sugar's Kyrity process uses the resin deposited on kieselguhr and does result in a pH depression in soft liquors to between 4.0 and 5.0.

A recent Japanese patent (Tanabe Seiyaku Co., Ltd. 1983) describes a process for removing iron from sugar liquors by gallo-tannin covalently bonded to an insoluble hydrophilic polymer, aminohexylcellulose activated with epichlorohydrin. The description is of treatment of a 20°Bx sugar solution, which implies that the technique has not been used outside the laboratory.

Tannin (tannic acid) is moderately toxic and precipitates proteins. It is nevertheless classified by the FDA as a GRAS "natural extractive" (generally recognized as safe). However a covalent derivative is a new substance, which presumably would require clearance if it were to contact food.

Mentioned earlier was the use of magnetic separators to reduce iron levels in char. Scale removal from metal surfaces should also be beneficial. We have had varying degrees of success in the laboratory with other techniques:

Chelating Resins - Iron can be removed quantitatively with resins such as Amberlite IRC-718, which might be visualized as immobilized EDTA, although the actual structure appears to be proprietary. Unfortunately, the resins do not have FDA approval for contacting food.

Iron-free liquors have an attractive golden color, whereas minute amounts of iron contamination result in a greenish hue. Spectrophotometric analysis showed that iron removal shifts the visual color to shorter wavelengths, from blue-green to yellow-orange; i.e., the absorbed color shifts from red to blue.

Ion Exchange Resins - Ferrous iron may be removed by cationic resins. The problem here is reducing iron to the ferrous form using an FDA approved additive that does not adversely affect flavor or appearance.

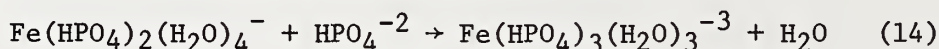
It is generally recognized that most decolorization on strongly basic polystyrene resins--except at high pH--is on the matrix or backbone of the resin, not at the functional groups. This is due to the relatively high electron density of the aromatic structure, which is attractive to non-polar and electrophilic species. Most colorants are best adsorbed at low pH, where they approach their isoelectric points. However functional groups must affect the nucleophilicity; otherwise cationic resins would decolorize equally well. (Phenolics sensitive to alkali may be picked up in their acid forms on cationic resins.)

We found that iron removal on decolorizing resins is not efficient, perhaps because the resins are not particularly good at removing phenolics (Kennedy and Smith 1976), which are the non-sugars most likely to contain complexed iron. Inhibition of complex removal could result if the occupied ligand sites on the colorant were also the locus of resin attachment. Moreover, soft liquors would quickly exhaust the resin.

Phosphate Addition - Phosphate somewhat facilitates removal of iron on both char and cationic resin, but the quantitative reproducibility of the effect is poor. A polyvalent cation may be favored, such as $\text{Fe}(\text{H}_2\text{PO}_4)_3$. Although one associates the species H_2PO_4^- with moderately low pH, it can be shown that even at pH 7,

$$\frac{\text{HPO}_4^{2-}}{\text{H}_2\text{PO}_4^-} = 0.62.$$

Added phosphate also results in a small but significant improvement in removal on anionic resin, again suggesting enhanced conversion to a polyvalent ion, such as



If phosphate is to be added, Na_2HPO_4 is probably a good choice, because acidic forms could compound the problem by solubilizing contact oxides.

In the granulated system, the addition of complexing agents will increase the fraction of the iron that ends up in the syrup rather than in the crystal. In soft sugar the relation is clearly far more complex.

pH Adjustment - The negative logarithm of the ferric ion concentration can be described in terms of pFe^{+3} , a quantity analogous to pH. It can be shown that

$$\text{pFe}^{+3} = -\log K_{\text{sp}} + 3 \log K_w + 3\text{pH} \quad (15)$$

where K_{sp} is the solubility product of the hydroxide. Hence

$$\text{pFe}^{+3} = 3 \text{ pH} - 6.04 \quad (16)$$

Just as high pH corresponds to a low $[H^+]$, so a high pFe^{+3} indicates a low $[Fe^{+3}]$. It can be seen that as the pH rises, the concentration of ferric ion that can be sustained in solution without precipitating the hydroxide becomes exceedingly small. This is why antacids interfere with absorption of dietary iron. At the same time, however, the stabilities of the complexes display an increase with increasing pH. Nevertheless, at pH 9, even the EDTA chelate decomposes.

We found that if soft liquors are brought to pH 8 and filtered, much of the iron can be removed as the hydroxide. Unfortunately this benefit must be weighed against the rapid generation of new color at pH 8.

Interestingly, removal of the precipitated ferric hydroxide, although it can form a remarkably stable sol, can be accomplished readily by filtration, at least in the laboratory. Addition of anionic polymer does not facilitate removal, despite the positive surface charge. The hydroxide is isomorphous with gelatinous aluminum hydroxide but is only slightly amphoteric.

Immobilized Phosvitin - Because of our toxicity concerns with reference to a tannin-based synthetic iron adsorbent, we set out to prepare a tannin-free experimental substitute. We chose to exploit the high affinity of phosphate for iron by utilizing phosvitin, a phosphate-rich (10% P) egg yolk protein which functions to carry phosphorus and associated calcium to the embryo chick. The phosphoprotein contains 100-120 phosphate groups, in clumps of phosphoserine residues, per molecule of 35,000 daltons.

An inorganic carrier was used. These are attractive because they are not subject to microbial attack and do not change configuration over an extensive pH range. A sample of phosvitin was immobilized on porous zirconia-coated alkylamine glass beads using glutaraldehyde coupling (Ramesh and Singh 1980). Glutaraldehyde reacts principally with amino groups in lysine residues. Although some lysine groups are undoubtedly phosphorylated, this is less common than at the serine and threonine sites, so that the iron capacity of the protein should not be seriously impaired by immobilization.

We found that the capacity of the immobilized phosvitin for iron can be enhanced by acid treatment prior to use, because the protein has an extraordinarily high iron content, 0.32%, almost exactly the level in mammalian hemoglobin. Many of the phosphate binding sites in the natural protein appear to be already occupied by iron.

The experimental adsorbent did an excellent job of iron removal, even when tested on liquors of high iron content.

Regeneration is effected with dilute hydrochloric acid. It can be calculated that if all of the phosphoseryl residues were available for iron adsorption, the adsorbent would have an impressive iron capacity of 18% of the protein mass.

A critical economic question here is how much purification of egg yolk would be required to isolate the fraction of phosvitin and similar phosphoproteins. Studies of adsorbent stability and bead size optimization have not been performed.

Microbiological Techniques - Special methods may be applicable to low density sources of iron. We can't resist including recent work by bacteriologists at MIT, Oxford, and the University of New Hampshire, who report that the bacterial species Aquaspirillum magnetotacticum takes iron in solution and concentrates it by a factor of 40,000 as magnetite inside its cell wall.

ANALYTICAL METHODS

Although we have found atomic absorption to be unreliable for iron, various colorimetric methods are available. Because highly colored soft liquors are likely to be unsuitable for colorimetry, we generally ash samples before analysis, using concentrated sulfuric acid. Do not be misled by the color of the ash: a reddish brown ash may not necessarily contain more iron than a less highly colored one. Kieselguhr may interfere; the silica appears to form an acid-insoluble inclusion compound with iron during ignition.

The ashed sample contains only the ferric form and is suitable for determination using ferrocyanide or thiocyanate. The Prussian blue reaction product with ferrocyanide is extremely stable, thus reducing the error by other complexing species. Thiocyanate can reduce ferric ion, but the difficulty can be circumvented by extracting the complex with isoamyl alcohol containing hydrogen peroxide.

We also use the highly sensitive bathophenanthroline procedure, particularly for granulated sugar. In the presence of high levels of phosphate, formation of the iron-bathophenanthroline complex is only about 2/3 complete, even after heating for two hours on the steam bath (Lohman et al. 1959). However a separation of iron from phosphates can be achieved using an iso-propyl ether extraction.

Citrate, oxalate, and lactate can interfere with most iron determinations, but these anions--unlike phosphate--will not survive ashing.

Generally interferences are a problem only at high concentration. Assume, for example, the dissociation constant for the thiocyanate complex is about 0.1 that of the phosphate complex--

not an immense difference. It can be shown that even if $[\text{PO}_4^{-3}] = [\text{SCN}^-]$, which is highly unlikely, 90% of the iron will nevertheless be present as the thiocyanate complex. Hence even under such extreme conditions, the error is only 10%. Note that the concentration of iron does not influence the result, only the ratio of competing complexing agents.

It is frequently useful to determine phosphate as well as iron. One method we have used successfully involves the addition of excess lanthanum, measurable by titration with fluoride using a fluoride electrode (Orion Research, Inc. 1971). High calcium levels interfere and require pretreatment with cationic resin.

A number of colorimetric methods using molybdate are available, some of which are impressively sensitive. We prefer the stannous chloride-in-glycerol version because of the stability of the reagent. If highly colored samples are ashed prior to analysis, the orthophosphate is converted to polyphosphate. However the P-O linkages in the latter are rapidly ruptured by heating the ash briefly to boiling in 1N acid.

SUMMARY

Iron contamination in soft sugar can adversely affect product quality and color stability. Oxide scale includes a removable ferromagnetic component, but rust is wholly paramagnetic.

We demonstrated the effect of iron in darkening to be purely catalytic. Consequently the oxidation state may not be particularly important in considerations of darkening rate but much more significant in elimination strategies. Particularly pertinent here is the high tendency of ferric ion for complexation, a result of its Lewis acidity. Removal may be a formidable task because of the high affinity of iron for other non-sugars. Moreover, iron-free liquors can readily pick up new iron.

There is considerable evidence for phenolic involvement in autoxidative darkening in soft sugars during storage. The roles of phosphate, pH, and light are addressed in the context of color formation.

Our progress in iron removal is described. Techniques studied include the use of adsorbents, and methods to modify complexes to more manageable forms.

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DISCUSSION

Angus Cameron, Norit: Thank you, Richard, for a very interesting paper. Just one question I would like to ask you. You said most of the iron comes from the refinery?

Riffer: Well, I would say that essentially everything that comes in with the raw sugar is removable. So, if you've got high levels of iron originating in the refinery, it might reach a point where it becomes unmanageable.

Cameron: From refinery to refinery, we find different iron levels in sugars.

Riffer: I expect that you would because some refineries have lined char filters. Phosphatation refineries could have higher levels because phosphate catalyzes oxidation of Fe^{+2} to the more difficult to remove Fe^{+3} .

Mary An Godshall, SPRI: I have a few comments, Richard. First, I'd like to compliment you on your interesting paper. You found that iron is quite important and we became interested in it from the dual points of view of flavor and color. I don't have any questions, but thought I would add a few of our observations. In flavor, we find that iron has two different flavor effects. One is that ferrous (Fe^{+2}) does taste metallic; the ferric (Fe^{+3}) is acidic. So the fact that a high iron content in sugar doesn't give a metallic flavor could be due to the form it's in. I don't know about the hydroxides and all these other forms you mentioned--I have no idea what they taste like. When we were doing some crystallization work, addition of ferrous sulfate to a white sugar solution produced a slightly green sugar, even though we felt that there were probably no phenolics in this white sugar.

Riffer: Ferrous iron itself is green, of course. The hydrated form could reside largely on the crystal surfaces.

Godshall: Yes, but the original solution was clear and we added only a few ppm. The other thing I wanted to point out was about the effects of iron on color. You mentioned the catalytic effect on color formation. There are also large color effects from iron complexing with phenols. A whole range of colors is formed, as you know. Phosphoric acid in very low concentrations breaks these complexes.

Riffer: We found that the phosphate complexes are less stable than the catechol complexes, but nevertheless, one can form them preferentially with a high enough phosphate concentration. The colors of the iron-phenol complexes depend upon the stability of the adduct, which is highly influenced by pH.

Michael J. Fowler, Amstar: I have found that iron concentration in liquid sugar is extremely important to color stability. If it goes above a certain percentage, your color will darken much more rapidly than it would with low levels of iron. We found that the source of the iron came from the sugar manufacture, and not the sugar itself, but from the Kieselgur, the diatomaceous earth, and sometimes the phosphoric acid.

John Lopez-Ona, Colonial Sugars: My previous employer, when I was in Philadelphia, used to adjust the soft liquor to pans by increasing the pH as you suggested, but we faced a problem doing that. Basically, the soft sugars we were making had a pH above 6.5, and some customers did not want pH's on soft sugars of 6.7 and 6.8. Of course, we tried to get an answer as to why this was a problem for them, and we never had an answer from the customer himself. Do you know why some of the users will refuse to accept pH's above 6.5?

Riffer: I think you're more likely to precipitate all kinds of inorganics and likely to generate new color at a much faster rate at a higher pH.

Lopez-Ona: Well, anything that precipitates would be taken off by filtration, I hope. I say the high pH's of liquor going to the pan gave me a high pH soft sugar compared to the other boiling scheme we had where the pH's were about 6.2 or 6.3.

Riffer: You may not necessarily be able to filter off everything because you could be forming colloidal ferric hydroxide which is extremely difficult to get rid of. It can be very resistant to flocculation.

Lopez-Ona: Then when the customer uses those soft sugars, he could have had trouble with some of those precipitates you are talking about?

Riffer: It's possible. Also, if lime is used to adjust the pH, calcium salts of organic non-sugars could precipitate.

Lopez-Ona: Thank you very much.

Robert Kunin, Consultant: Have you ever looked at the old phenolic ion-exchange resins for removing iron?

Riffer: I don't think we've looked at that recently.

Kunin: They're still available, and they should work, and they are FDA approved. They are being used in the corn sugar industry, and I wonder how they would work on removing the iron from these soft sugars.

Riffer: It seems to me that we studied them. I don't think they did a particularly good job, perhaps because the iron is present in very stable complexes with non-sugars--more stable than the resin-iron complex, perhaps. The kinetics could also be unfavorable, if the iron is complexed with large molecules.

ANALYTICAL METHODS OF MEASUREMENT--A NEED FOR CORRESPONDENCE

Stephen A. Brooks and R. A. Melanie Pilgrim

Sugar Technology Research Unit, Barbados

INTRODUCTION

Analysts perform determinations on a wide range of substances by an ever increasing range of instruments having greater and greater sensitivity. In a recent comparison on analytical progress, Professor Allen Bard (1983) of the University of Texas noted that when the National Academy of Sciences Westheimer report was published in 1965, concentrations in ppm, or even occasionally ppb, were measured. Today, with improvements in instrumentational sensitivity, analysts can sense concentrations at the ppt level. However, because of the diversity of analytical methods available to us today, a new problem raises its head, namely that of correspondence between different methods of analysis. The need to prepare samples such that all methods of analysis monitor the same parameter, becomes of paramount importance.

This paper sets out to show that in the case of calcium determination of process liquors in a raw sugar factory, it is necessary to implement a pretreatment of the sample before performing the analysis using atomic absorption spectrometry, in order that results agree with those obtained by EDTA or ion selective methods of analysis. An awareness of the existence of a problem arose when attempts were made to streamline analytical procedures during recent trials on evaporator scale prevention using chemical means.

During the course of this trial various process liquors were initially analyzed for calcium using atomic absorption spectrophotometry on the untreated sample. The results of these analyses were not consistent with a visual inspection of the evaporator tubes, i.e. the mass balance calculation suggested that "tons" of calcium were being produced, while visual inspection showed only a slight deposit. It was therefore decided to determine the calcium levels in these process liquors by five different methods of analysis in

addition to the standard EDTA complexometric titration method. The five procedures were compared statistically to the EDTA results.

The main constituents of evaporator scale are listed in Table 1. These consist of the phosphates, sulphates and silicates of calcium, magnesium, iron and aluminum, together with the salts of oxalic and aconitic acids. The composition of these substances varies from evaporator vessel to evaporator vessel and from factory to factory. Springer (1953) suggested that in cane juices undergoing concentration, a negatively charged intermediate colloidal complex could exist during saturation and supersaturation. Springer's suggestion also appears to support the view held by Honig (1950). Furthermore, Honig also listed the source of phosphate levels in mixed juice and clarified juice as originating from protein phosphate, lipoid phosphate, and inorganic phosphate, thus indicating different types of phosphates. The object of this procedure is to get the calcium salts into a form which will enable them to be quantitatively analyzed. Bennett (1956) clearly states the theory for calcium solution using EDTA, but as our work used oxalic acid as a source for the predigestion, that theory will be outlined here.

Table 1.--Groups found in evaporators of raw sugar factories

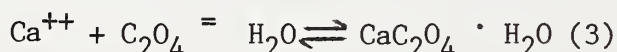
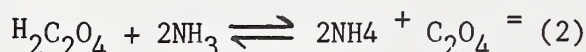
Silica	(SiO ₂)
Magnetite	(Fe ₃ O ₄)
Calcium salts of sulphuric, phosphoric and carbonic acids	
Calcium oxide	(CaO)
Magnesium hydroxide	(Mg(OH) ₂)
Zinc oxide	(ZnO)
Copper oxide	(CuO)
Alumina	(Al ₂ O ₃)
Organic acids (oxalic and aconitic acids)	

Theoretical considerations

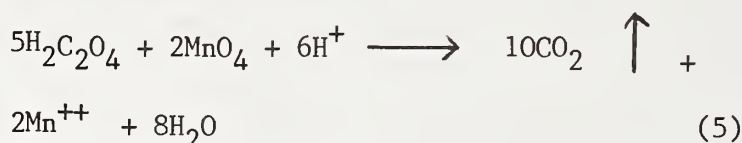
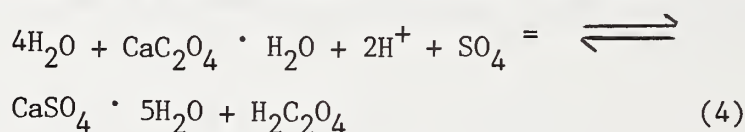
Calcium is precipitated as calcium oxalate, CaC₂O₄·H₂O by treating a hot hydrochloric acid solution with ammonium



oxalate. No precipitation of CaC_2O_4 occurs at this stage as the solubility product of CaC_2O_4 is not exceeded. The solution is slowly neutralized with aqueous ammonia to a pH of 4.0, when CaC_2O_4 precipitates quantitatively. MgC_2O_4 , although insoluble, remains in the supersaturated state and does not precipitate provided the digestion time is kept short.



The calcium oxalate precipitate is washed sparingly with hot distilled water, since it is only moderately insoluble. The washed CaC_2O_4 is metathesized with dilute H_2SO_4 and the liberated $\text{H}_2\text{C}_2\text{O}_4$ is either titrated with KMnO_4 or the calcium is analyzed by atomic absorption spectroscopy.



MATERIALS AND METHODS

Appendix 1 lists the procedures used in this study.

Atomic absorption (AA) spectroscopy was performed on a Perkin Elmer model 107.

RESULTS

Table 2 lists the results obtained by the six methods. The results shown are the average of triplicate determinations. Table 3 lists the correlation coefficients and coefficients of variation of both the clarified juice and the syrup. Both sets of figures for the correlation coefficients were obtained by statistically comparing the various methods to the EDTA results. The correlation coefficient can be used to test whether the relationship between one set of data and another is a linear one. Deviations from unity indicate the possibility of interference.

Table 2.--Calcium concentration determined by six methods

Sample Number	EDTA Titration	Ion Se- lective	ppm calcium		Oxalate KMnO ₄	Oxalate A. A.
			A. A. Ashed	A. A. Unashed		
1	322	336	353	361	292	314
2	347	471	319	396	389	350
3	388	489	459	423	418	371
4	360	459	366	346	362	353
5	337	402	361	344	347	316
6	380	533	391	394	387	323
7	401	476	433	492	461	354
8	338	428	366	394	372	311
9	382	493	370	369	411	382
10	377	471	371	419	403	396
11	1548	1792	1443	2832	1801	1505
12	1607	1567	1565	2158	1695	1382
13	1681	1672	1385	2563	1905	1503
14	1967	1888	1612	2921	2092	1808
15	1485	1497	1307	2274	1692	1397
16	1320	1532	1133	2001	1684	1407
17	1519	1595	1392	2311	1686	1410
18	1794	1808	1579	2721	1899	1719
19	1640	1726	1509	2554	1893	1607
20	1537	1925	1457	2550	1950	1699
21	1768	1930	1607	2467	1970	1643

Samples 1 - 10 are clarified juices.

Samples 11 - 21 are syrups.

Table 3.--Paired t-test on calcium methods

Method (Juice)	Corr. Coeff./EDTA	t-value (t 0.025, n9=2.26)	Signf. Diff. at 5% or 10%	
			<u>5%</u>	<u>10%</u>
Ion Selective	0.8152	7.91	Yes	Yes
AA Ashed	0.7216	1.78	No	No
AA Not Ashed	0.6397	3.098	Yes	Yes
Oxalate Titr.	0.8975	2.67	Yes	No
Oxalate AA	0.6748	-2.22	No	No
(Syrup)		(t 0.025, n10=2.23)	<u>5%</u>	<u>10%</u>
Ion Selective	0.6475	2.28	Yes	No
AA Ashed	0.8514	-6.15	Yes	Yes
AA Not Ashed	0.7291	14.62	Yes	Yes
Oxal. Titr.	0.8152	7.13	Yes	Yes
Oxal. AA	0.7667	-2.08	No	No
For 10% t 0.01, n9 = 2.82 t 0.01, n10 = 2.76				

The correlation coefficient (r) is expressed by the formula:

$$r = \frac{\sum_{xy}}{\sqrt{S_x^2 S_y^2}}$$

$$\text{where } S_{xy} = \sum_i x_i y_i - \frac{(\sum x_i)(\sum y_i)}{n}$$

$$S_x^2 = \sum_i x_i^2 - \frac{(\sum x_i)^2}{n}$$

$$S_y^2 = \sum_i y_i^2 - \frac{(\sum y_i)^2}{n}$$

for n, the number of samples, x the EDTA results and y the results obtained by the other analytical method.

The actual values of the calcium concentrations obtained by some of the different methods differ significantly from the EDTA standard (in particular ion-selective determinations for juices and A.A. unashed for syrups). But the data does not

show any correlations differing significantly from unity. However, the sample sizes are small and similar correlations with 50 to 100 observations would be significantly different from unity.

A better test for comparing the results for equivalency is the paired t-test

$$\text{where } t = \frac{\bar{d}}{S_d/\sqrt{n}} \quad \text{for } \bar{d} \text{ the}$$

averaged difference for two determinations, S_d is the standard deviation of the observed differences and n is the number of pairs under study. Table 3 shows that Oxalate AA showed no significant difference from EDTA for either juice or syrup determinations, while AA ashed showed no significant difference for juice.

The results are represented graphically in Figures 1 and 2. The separation of untreated samples (AA unashed) from treated samples is especially clear in the syrup samples (Figure 2). The graphs also underline the effectiveness of using ion selective analysis on untreated samples as a quick method of determining the calcium levels of syrups. On this point, it should be noted that ion selective analysis using the sample addition method was performed on the untreated sample, because of the low pH of the treated sample. In this method, a Philips IS-561 calcium electrode was used and measurements were made in the concentration range of 0.04 - 40,000 ppm and in the pH range of 3-12.

In addition, the graphs indicate that the syrup analyses by ion selective analysis more closely follow the bulk of methods used, while the clarified juice results by this method appear somewhat adrift. A possible explanation for these unusual results may lie in the respective magnitudes of ΔE . ΔE syrup was between 26.2 and 29.3 MV which was an acceptable sensitivity for this type of selectrode, while for the juice, ΔE values ranged from 18 to 22.8 MV - i.e. approaching an insensitive region of this electrode and therefore contributing to the error.

CONCLUSIONS

This paper, it is hoped, will be used as a forum to generate work on the chemical nature of solutions found in evaporators, with a view to solving problems which pertain to evaporator efficiency, before scaling results. In addition, it makes us aware of the need to standardize all methods of analysis so that results may be readily compared.

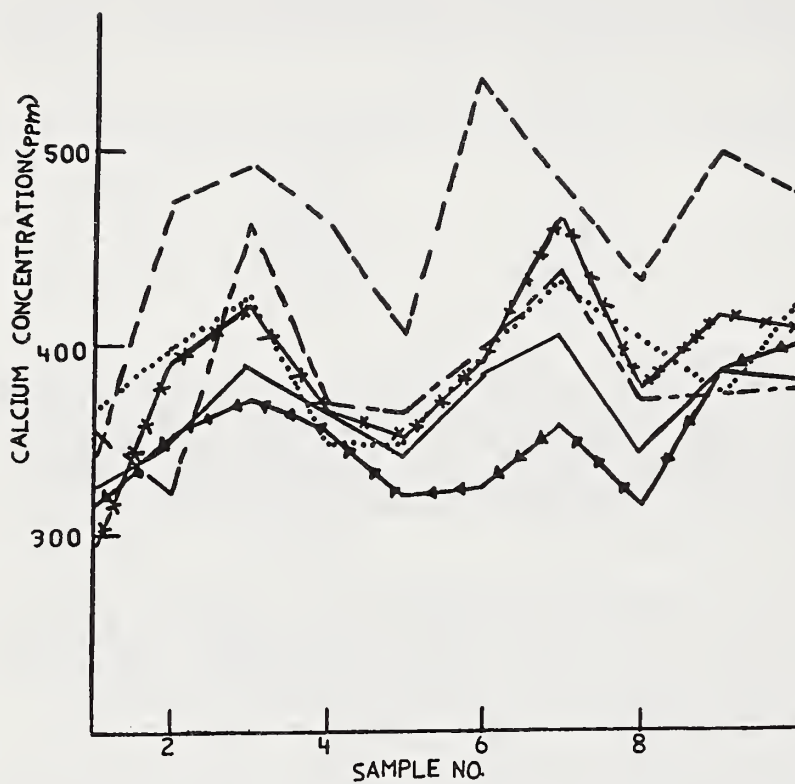


Figure 1.--Graphic depiction of calcium in clarified juice by 6 methods of analysis. —, EDTA complexation; ---, ion selective analysis; —▲—, AA ashed, AA unashed; —***—, oxalate - KMnO_4 titration; —x—, oxalate-AA.

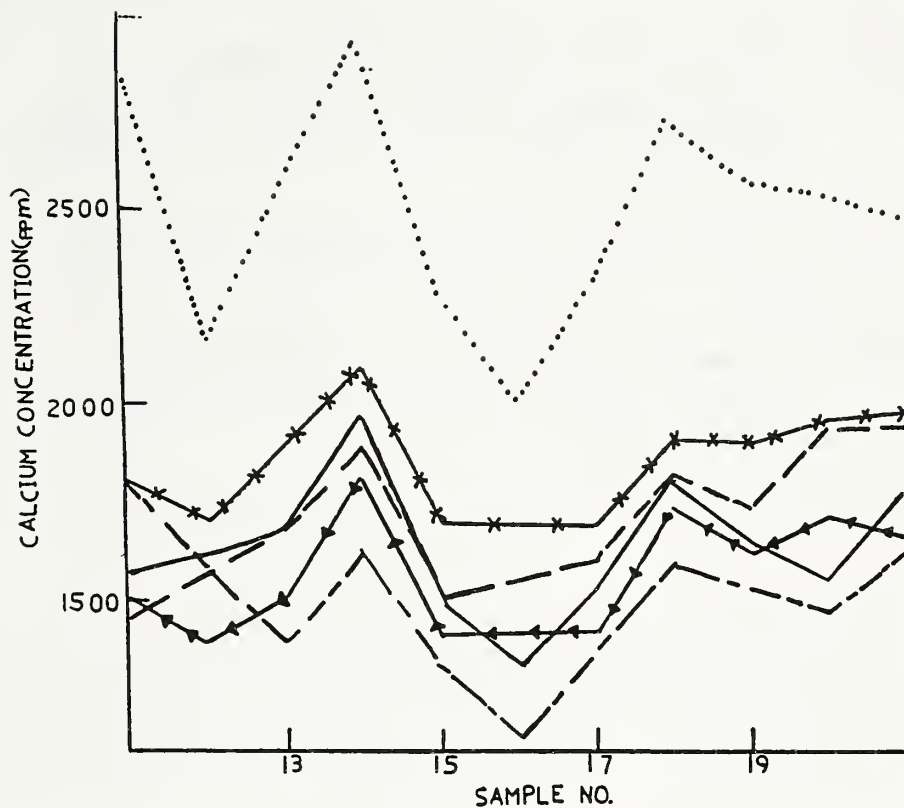


Figure 2.—Graphic depiction of calcium in syrup by 6 methods of analysis. —, EDTA complexation; —, ion selective analysis; —, AA ashed, AA unashed; —***—, oxalate - KMnO_4 titration; —x—, oxalate-AA.

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APPENDIX I

- A. Procedure for Ashing
- B. EDTA Titration
- C. Preparation of Calcium Oxalate and Permanganate Titration
- D. Ion-Selective Analysis
- E. Oxalate Precipitation/AA analysis

A. Procedure for Ashing Reagents

1:1 Sulphuric acid
Concentrated hydrochloric acid

1. Clarified Juice

The juice (40 g) was weighed in a 100 ml beaker. A small silica dish was weighed and placed on the water-bath. The juice and beaker washings were transferred to the dish and this mixture evaporated to a thick syrup. Sulphuric acid (2 ml of 1:1 acid) was added and the syrup evaporated to the charring point on a hotplate.

The dish was then placed in the muffle furnace at 550° C and the temperature allowed to rise to 800° C over a period of one hour. The dish was removed from the furnace, cooled, and a further aliquot of sulphuric acid added (1 ml). The dish was heated on the hotplate until all acid fumes were driven off; after this period it was returned to the furnace for one hour, at a temperature of 800° C.

The resulting ash was cooled and dissolved overnight in concentrated hydrochloric acid (10 ml). The solution was made up to 100 ml.

2. Syrup

The syrup (15 g) was weighed directly into the silica dish. Sulphuric acid (5ml of 1:1 acid) was added and the sample heated to dryness. The dish was placed in the furnace at 550° C until no black color remained in the sample. The dish was removed and cooled, and sulphuric acid (3 ml of 1:1) added to the residue. After this was heated to dryness on the hotplate, the dish was once again placed in the furnace at 800° C for thirty minutes. The ash was treated as in A.

B. EDTA Complexometric Titration for Calcium Determination

Reagents:

Standard EDTA solution, 4%
Hydroxylamine Hydrochloride, 5%
Potassium Cyanide, 3.5%
Potassium Hydroxide, 45%
Monoethanolamine Buffer
Calcium/Magnesium Indicator
Calcium Standard, 1000 ppm
Calcium Indicator
Ashed solutions of clarified juice and syrup

Procedure:

i) Standardization of EDTA solution

Standard calcium solution (10 ml of 1000 ppm) was pipetted into a 250 ml conical flask and distilled water (40 ml) was added. Hydroxylamine hydrochloride (3 ml) and potassium cyanide (5 ml) were added. A small piece of red litmus paper was inserted and the solution made just alkaline with potassium hydroxide. Monoethanolamine buffer (5 ml) was added and enough Ca/Mg indicator to give a bright pink color. The solution was properly mixed and titrated with standard EDTA with constant stirring to a blue end-point.

ii) Titration of Samples

A 20 ml aliquot of the solution of acid soluble ash (prepared in the "Procedure for Ashing") was pipetted into a 250 ml conical flask. Hydroxylamine hydrochloride (3 ml) and potassium cyanide (5 ml) were added. A small piece of red litmus paper was dropped into the conical flask and the solution made just alkaline with potassium hydroxide; excess hydroxide was then added (4 ml). The solution was mixed thoroughly and a small scoop of calcium indicator added. The test solution was titrated with standard EDTA solution to a blue end-point.

C. Preparation of Calcium Oxalate and Permanganate Titration

The sample (15 g syrup; 40 g clarified juice) was weighed in a scoop, transferred to a 250 ml beaker, and made up to approximately 130 ml with distilled water. Glacial acetic acid (5 ml) and a teaspoonful

of kieselguhr were added; the mixture was brought to boil and then boiled for five minutes. The hot mixture was filtered through Whatman No. 1 paper into a 400 ml beaker. The original beaker and filter paper were washed three to four times with hot distilled water and the washings also collected in the 400 ml beaker.

Concentrated hydrochloric acid (2 ml) was added and the solution heated once again to boiling. Saturated ammonium oxalate solution (25 ml) was added and the acid solution neutralized with 6M ammonia solution. The end-point, at pH 4, was identified by using universal indicator paper. Fine particles of calcium oxalate came out of solution at this time.

After thirty minutes, the mixture was filtered through Whatman No. 40 ashless paper. The precipitate was washed three times with hot distilled water. The filter paper and precipitate were transferred to a beaker containing 100 ml of water and 10 ml of 1:1 sulphuric acid. The contents of the beaker were brought to 90° C, then allowed to cool to 60° C, with constant stirring. The titration was started when the temperature reached 60° ; 90% of the permanaganate was added immediately. The titration was completed by drop-wise addition of permanaganate. The end-point was taken to be the first pink color lasting for thirty seconds.

D. Procedure for Ion-Selective Analysis Reagents

Calcium standards
4M KCl-ionic strength adjuster (ISA)
1M KCl-outer filling solution, reference electrode
Saturated KCl-inner filling solution, reference electrode
Clarified juice, 1.0 ml aliquots
Syrup, 0.5 ml aliquots

Procedure

The method used was "Sample Addition." This was rapid and required very little sample solution. As a preliminary, the electrode sensitivity, A, was determined. S is defined as the millivolt (mV) change per decade change in ion concentration. Therefore, solutions of 1, 10, 100 and 1000 ppm calcium were prepared. The mV reading of 50 ml of solution plus 1 ml of ISA, was measured and recorded. This

was repeated for each standard solution after which a graph of mV versus log concentration of solution was plotted, and S determined by calculating the slope of the line.

The ion selectrode was immersed in 100 ml of 1 ppm calcium standard containing 2 ml of ISA. After 2 minutes, a mV reading was taken; then at 2.5 and 3 minutes, when an aliquot of sample was added. Millivolt readings were again noted at 5, 5.5 and 6 minutes. In this way, triplicate readings were obtained. The difference in millivolt readings, mV after addition - mV before addition, gives ΔE . The latter was used to calculate the concentration of sample by the following equation:

$$C_x = C_{st} \cdot \frac{V_{st}}{V_x} \left[10^{\frac{\Delta E}{S} \left(\frac{V_x}{V_{st}} + 1 \right)} - 1 \right]$$

Where C_x = concentration of sample (moles l^{-1})
 C_{st} = concentration of standard (moles l^{-1})
 V_{st} = volume of standard
 V_x = volume of sample
 ΔE = millivolt change
 S = electrode sensitivity, mV change/decade
 conc. sensitivity, mV change/decade conc.

E. Procedure for Oxalate Precipitation/AA analysis Reagents

Concentrated hydrochloric acid
 Concentrated sulphuric acid
 1:8 sulphuric acid
 Saturated ammonium oxalate
 6M Ammonia
 5% Lanthanum solution
 Calcium standard (1000 ppm)
 Filter aid
 Universal indicator paper
 Whatman No. 40 paper, ashless
 Whatman No. 1 paper
 Clarified Juice, 40-70 g
 Syrup, 15 g

Procedure

The sample was weighed in a scoop and transferred to a 250 ml beaker. The volume was brought up to 130 ml with distilled water and 5 ml of conc. hydrochloric acid added. The mixture was brought to a boil. Syrups were boiled for one minute, clarified juices for five minutes. A scoop of filter aid was added to clarified juices.

The clarified juice was filtered through Whatman No. 1 paper into another beaker. The first beaker and filter paper were washed three or four times with hot, distilled water. To this hot filtrate, or the boiled syrup mixture was added 30 ml of hot, saturated ammonium oxalate and the pH brought to 4, by dropwise addition of 6M ammonia.

After 30 minutes, when precipitation was complete, the calcium oxalate was filtered onto Whatman No. 40. The beaker and paper were washed once with hot, distilled water, then dissolved in 1:8 sulphuric acid and conc. sulphuric acid. The mixture and washings were transferred to a volumetric flask. This solution was diluted, with addition of 5% lanthanum, to be analyzed on the AA.

ELECTROSTATIC METHODS TO SEPARATE BONE CHAR FROM GRANULAR

CARBON: PRELIMINARY REPORT

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INTRODUCTION

Bone char in its granular form was first used to refine sucrose in 1828 in Paris by a confectioner named Dumont. Before that time, bone char had been used only in powdered form and discarded after each use (Barrett, 1951). This innovation made possible the production of more and better quality refined sugar, and for well over a century following Dumont's discovery, granular bone char was the main decolorizing agent in all sugar production. The bone char house in the current design and scale became a regular part of a refinery in the late nineteenth century. A fine history of the use of bone char and other carbons has been written by Dr. Victor Deitz (1944).

Vegetable charcoal has been used as an adsorbent for several hundred years; activated carbon became popular for use in sugar refining about 1912-1915, pioneered primarily by the Norit Company in Europe. This material was also powdered but recovery and regeneration systems were frequently used and the adsorbent was recycled rather than discarded after each use.

Granular carbon, developed by the Pittsburgh Coke and Chemical Company in the 1940's, entered the market for sugar decolorizing adsorbents in the 1950's and found wide application in the following decades. The high temperature needed for its regeneration made it an alternative or supplement to bone char, requiring a high temperature furnace rather than the lower temperatures of bone char regeneration kilns. During these years, other adsorbents such as Synthad, a synthetic bone char (Barrett, 1951) came and went, but bone char remained the major adsorbent in sugar refining. The advantages of granular carbon, particularly its intense decolorization powers and small capital equipment requirement, did not overcome the familiar advantages of bone char's dual adsorption of organic and inorganic material.

In the 1970's, fuel crises hit, and fuel intensive processes were re-examined. The process and cost gains of using granular carbon in conjunction with bone char were very appealing, but the necessity for two regeneration systems was not. It was known that bone char should not be regenerated at higher temperatures than those in general usage (Carpenter and Deitz, 1961); could granular activated carbon be renewed at lower temperatures than had been thought necessary?

W. L. Reed (1979) at Revere Sugar Company found that granular carbon could be regenerated at lower temperatures than had been the practice and could still decolorize sugar liquors successfully. In 1981, a patent was issued to the Calgon Corporation, who now encompasses the former Pittsburgh Coke and Chemical Company, on the process of mixing granular activated carbon and bone char for decolorizing and deashing cane sugar liquors (Reilly, 1981). Several refineries in North America, Europe, Australia and New Zealand, and elsewhere have adopted the process, known as the Canesorb System, and reports on its use have been published (Barton and Knebel, 1982; George and Sulick, 1982). It has been shown that the granular carbon can be regenerated in traditional bone char kilns (George and Sulick, 1982; Zemanek, 1984).

As with any new development, new problems have arisen. There is a need to separate service bone char from granular carbon on a laboratory scale so that tests of regeneration efficiencies can be conducted, a process not required until this new system went into operation.

In addition, because bone char and carbon differ greatly in their physical properties of hardness, attrition rate, and regeneration temperature, the loss of one component, i.e., carbon, at a greater rate than the other, is to be expected during service and regeneration. A method is required to determine what percent of granular carbon remains in the admixture after regeneration so that the loss can be made up. This method must be practical; that is, rapid, simple, inexpensive and accurate.

At present, a water separation procedure is employed to determine the percentage of components in a bone char-carbon mixture. In this method, the mixture is placed in a column and water is pumped upward through the adsorbents, fluidizing the bed. The upper layer is presumed to be carbon and the lower layer char. The majority of the material in the middle of the column is a mixture, which is discarded, and the upper and lower cuts are analyzed.

This method has some inherent problems. Bulk density and particle size are the major contributing factors in a water separation. Since bone char and carbon particles have a range over which their size and density overlap, there is a carry-over of one component into the other. Most especially, one would expect char particles

to contaminate the carbon layer, and only the heavier and larger char particles would accumulate in the bottom layer.

Char and activated carbon also differ in their chemical composition. Carbon is composed of 83 to 88% C while bone char is 8 to 9% C and about 90% calcium phosphate in various forms. Because of these differences, it may be assumed that the electrical properties would also differ.

Separation by Electric Fields.

Separation of components by A.C. electric fields is a novel concept. Masuda and Matsumoto, in 1974, developed an electric grid covered with a dielectric sheet to study the ability of an electric field to levitate and transport powders. They found that some powders were "active", that is, could be manipulated by the field. Among these active powders were polyethylene, epoxide resin, and alumina. Conductive powders such as metal and activated charcoal were violently ejected. By contrast, some powders were found to be "nonactive", and showed no sign of being moved by the electric field. These included nylon and PVP powders. This phenomenon was found to have practical application in the paint industry and was later patented (Masuda, 1975) as an apparatus for controlling the flow of particulate matter. He named this a "contact-type, electric curtain panel", but it is commonly referred to simply as an "electric panel" or Masuda panel.

The electric panel is a traveling-wave apparatus in which parallel sets of individual conductors are connected to a three phase A.C. source to form an undulating traveling wave electric field above the panel. Material placed within the field develops a charge and is transported along the waves in either a positive or negative direction off the panel, depending upon composition and electrical properties (Weiss and Thibodeaux 1984). With some materials, a sufficient charge is not developed, and these remain on the panel.

Figure 1 shows a photograph of the top surface of a Masuda panel. The conducting wires are embedded in a dielectric material, close to the surface. A schematic diagram of the wiring circuit of the panel is shown in Figure 2. The dotted lines represent the outline of the plastic panel. Grid space is the distance between electrical conductors. Here, it is 3.0 mm. Every third electrode is connected to one phase of the three-phase power supply.

During a separation, charged particles are levitated into the undulating electric field formed by the three phases. The more strongly charged particles move along the top of the field in one direction, while the less charged particles do not rise as high and move along the lower edge of the field in the opposite direction (Figure 3).

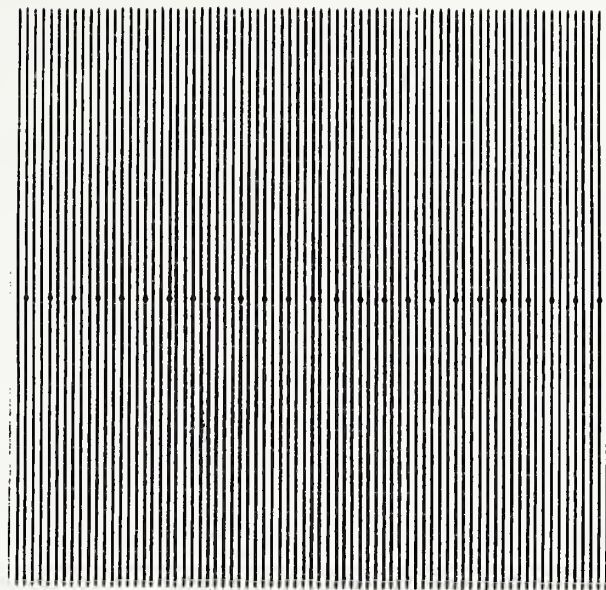


Figure 1.--Top surface of an electric panel showing placement of the conducting wires.

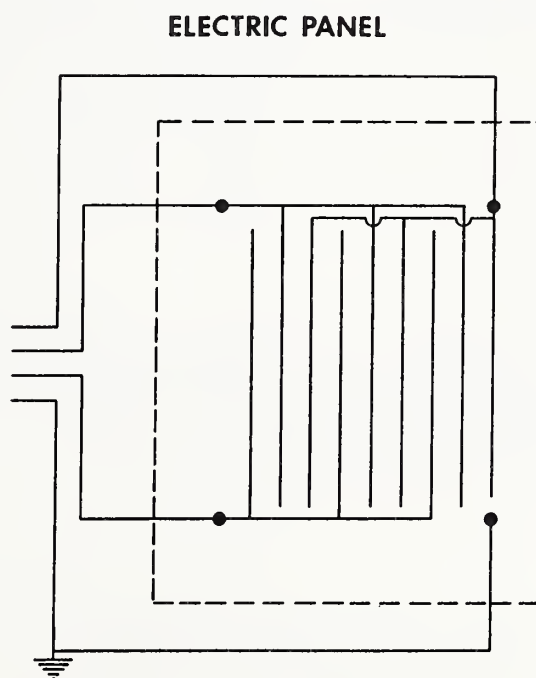


Figure 2.--Diagram of the wiring circuit of an electric panel with a 3-phase system. Dotted lines indicate panel outline.

Electric panels are able to transport textile fibers (Weiss, 1982), and have also been found to be able to separate and upgrade agricultural mixtures into a protein-enriched fraction and to remove objectionable components such as hulls, gossypol, plastic and mold contaminated flour (Weiss and Thibodeaux, 1984).

This report describes attempts to separate mixtures of bone char and carbon into their component parts in an A.C. electric field with a Masuda panel and in a D.C. electric field with various configurations of electrodes.

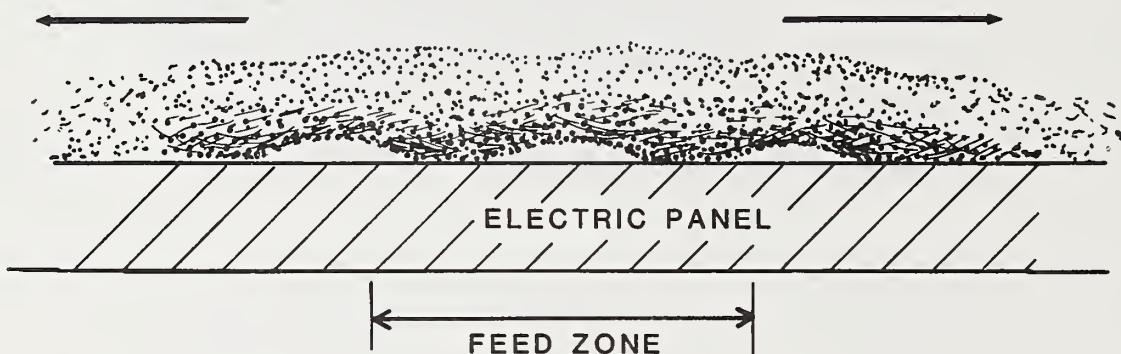


Figure 3.--Diagram of the theoretical distribution of particles on a charged panel. The less charged particles remain closer to the surface of the panel.

MATERIALS AND METHODS

Samples of bone char and granular carbon were obtained from 5 sugar refineries and a sample of granular carbon was also obtained from Calgon Corp.

Composition of the char and carbon samples was obtained using an Ortec Energy Dispersive x-ray fluorescence instrument.

A. C. separation. Small samples, about 1-5 g, of either bone char or carbon or mixtures of known composition were placed in the center of an electric panel and the voltage to the plate cycled several times from about 4000V to about 5000V until the particles would not move any more. During the run, the sample was periodically disturbed gently with a sable brush to stimulate charging action. A run lasted 2-5 minutes, depending on sample size. The panels were surrounded by cardboard collars to serve as catch basins for material ejected from the panels. Details of the A.C. circuit along with the specifications for construction of the electrical panels have been published (Weiss, 1982; Weiss and Thibodeaux, 1984).

Separation efficiency was calculated as the proportion of the starting material that was transported or not transported.

D.C. separation. Shaped electrodes, machined from aluminum or stainless steel, were placed 0.5-1.2 cm apart. A small sample of adsorbent was placed on the bottom electrode and a specified voltage applied, or else fed through a funnel to the bottom electrode while a D.C. voltage was applied. The electrodes received opposite charges. The simplest configuration of electrodes consisted of two flat aluminum plates, about 20 cm x 4 cm. More efficient configurations consisted of an upper ball electrode placed over disc-shaped or bowl-shaped lower electrodes.

Charge to mass ratio. The charge to mass ratio of bone char and carbon was determined using the electric panel. Carbon particles were charged at 5-6 kV and collected in a Faraday pail as they left the panel. The charge was measured by a Keithly electrometer Model 610B. The mass was weighed by analytical balance to obtain the ratio. Since char did not leave the panel after charging, it was gently swept into the pail and the charge determined as described above.

RESULTS AND DISCUSSION

Because of the differences in composition of bone char and carbon, it was originally hoped that x-ray fluorescence could be used to determine the char-carbon composition of mixtures separated by electrostatics by determining calcium and phosphorus concentration. The method is rapid, requiring no extensive sample preparation.

The x-ray results with admixtures were on occasion showed a wide variations in composition of the starting materials. Therefore, the separation efficiency was also determined as the weight percent of the separated fractions to the whole sample.

X-ray fluorescence was useful, however, in providing detailed information about the composition of trace elements in bone char and activated granular carbon. Bone char showed a lot of variation, which can be attributed to the varying proportion of carbon in its make-up and to different sources of supply. The comparative proportions of several elements in char and granular carbon, according to x-ray fluroescence, are listed in Table 1. The most significant compositional differences occur with calcium, which averaged 21.5% in bone char and 0.32% in carbon, and with phosphorus, which averaged 13.4% in bone char and 0.18% in carbon.

Table 1.--Composition of bone char and granular activated carbon by X-ray fluorescence

Element	<u>% of Component*</u>	
	Bone Char	Granular Carbon
Calcium	15.6 - 26.1	0.256 - 0.430
Phosphorus	8.96 - 17.23	0.018 - 0.296
Silicon	2.19 - 4.50	0.637 - 2.24
Iron	0.17 - 0.243	0.494 - 0.764
Aluminum	1.37 - 3.60	0.002 - 0.960
Potassium	0.29 - 0.476	0.098 - 0.186
Sulfur	0.34 - 0.669	0.620 - 1.53

* Range represented by 9 samples.

Separation on electric panels. Particles were charged by interaction with the plastic and were levitated according to the charge acquired per unit mass (q/m). The charges on materials for this investigation were determined in micro-coulombs per gram (uC/g). When the panel was energized at 5 to 6 kV the q/m ratios were as follows: Granular carbon, +0.94; and bone char, -0.001. The charge acquired by granular carbon was similar to that acquired by several proteins (Weiss and Thibodeaux, 1984), but the direction of travel was in both directions, unlike the proteins and Lycopodium (a fern spore used as a standard), which travel in only one direction (herein referred to as the Lycopodium direction). Most of the bone char did not levitate, even at the maximum applied voltage of 6000V, but remained essentially stationary on the panel.

The water content of these materials was measured by weight and expressed as percent moisture loss after drying. Carbon was 0.07% and char was 3.7%.

Table 2 summarizes the activity of carbon on an electric panel. The percentages reported were obtained as percent by weight. The results showed that most of the carbon (97.5%) was transported off the panel, 57.1% in the Lycopodium direction, and 41.3% in the opposite direction. A high relative humidity (70%) had a slight negative effect on transport.

Table 3 summarizes the results obtained when various samples of new and regenerated char were placed on the panel. The behavior of bone char was opposite that of carbon in that char remained on the panel. New char averaged 98.9% remaining on panel and regenerated char averaged 91.3% remaining on panel. Relative humidity over a range of 0 to 72% had little effect on the separation. The higher moisture content of the bone char may aid in holding the char to the panel.

Table 2.--Summary of the behavior of new carbon (Canesorb) on an electric panel*

Sample	% Remaining on panel	% L ** direction	% Anti-L direction	% Total off Panel
A2-1	0.6	59.5	39.5	99.0
A2-2	8.5	--	--	91.4
A2-3	1.9	56.0	42.2	98.2***
B2-1	1.4	59.8	37.2	97.0
B2-2	0.1	74.1	25.8	99.9***
C2	1.8	48.5	48.6	97.1
D2	0.5	54.5	44.3	98.8
E2-1	1.3	50.7	47.1	97.8
E2-2	0.6	53.4	46.0	99.4***
F2	4.0	--	--	96.0

% Off Panel	=	97.5 \pm 2.44	(2.51% C.V.)	n=10
% In L-direction	=	57.1 \pm 7.92		
% In anti-L	=	41.3 \pm 7.36		

*Results are reported as percent by weight of original sample.
 **L-direction is the Lycopodium direction. (See text)
 ***These samples were dried in a vacuum oven prior to separation.

Table 3.--Summary of the behavior of new and regenerated bone char on an electric panel.

New Char	% Remaining on Panel	% Moved off Panel
A1-1	98.8	0.60
A1-2	99.4	0.63
A1-3	99.4	0.60**
B1	97.5	1.79
B1/T16 (<1 mm)	97.6	--
B1/NT16 (>1 mm)	99.0	--
C1	99.7	0.27
D1	99.7	0.26
E1	98.3	--
Mean	98.8 \pm 0.85	
Regenerated Char	% Remaining on Panel	% Moved off Panel
A	87.3	5.5
B	92.6	7.4
C	93.9	5.2
Mean	91.3 \pm 3.50	
Over-all mean remaining on panel = 96.9 \pm 3.80		

**Sample dried in a vacuum oven.

T16 = through Tyler 16 mesh; NT16 = did not pass through Tyler 16 mesh.

Values are expressed as a percentage by weight of the original sample.

The results in Tables 2 and 3 demonstrated the potential this method had for separating these two adsorbents. The results were less promising when mixtures of granular carbon and bone char were placed on the panels and separated. Separation efficiencies for an 80:20 mix of char:carbon ranged from 24% to 88%, with a mean of 50.6%. The data are summarized in Table 4. In these separations,

there was some indication that transport of the carbon did not occur as effectively in the presence of bone char.

These results may indicate that an interaction occurs between the two adsorbents in the electric field so that there is neutralization of the charge on the carbon. They may also indicate that at this high concentration the char is shielding the carbon and physically preventing carbon movement. Possibly bone char, acting as an an insulator, prevents the charging of the carbon by contact with the panel surface.

Table 4.--Summary of panel results in separations of 4:1 mixtures of bone char and activated carbon

Sample	% Remaining On Panel*	%R.H.	Separation Efficiency (%)
C1 + Regen A	95.1	28	24.5
C1 + Regen B	95.2	28	25.0
C1 + Regen C	94.8	28	26.0
C1 + Regen D	82.7	--	86.5
C2 + New A SM	82.4	40	88.0
C3 + New B SM	84.3	41	78.5
C4 + New C SM	94.9	72	25.5

* To obtain a separation efficiency of 100%, the amount left on the panel should be 80%.

C1 to C4 = different batches of new Canesorb; Regen A to D = regenerated bone char from different refineries; New A SM to C SM = new bone char from different refineries sieved through Taylor 16 mesh.

X-ray fluorescence was useful in confirming that a carbon-rich fraction was expelled from the panel while a char-enriched portion remained on the panel. Mixtures containing 1:1 and 4:1 char:carbon by weight were separated on a panel, and the separated fractions (on the panel and off the panel) were analyzed for calcium and phosphorus content by x-ray fluorescence. The results are summarized in Table 5. Several conclusions can be drawn from these results: Significant enrichment of fractions occurs upon separation, but the first pass is not 100% efficient; X-ray fluorescence is not sensitive enough to distinguish a 4:1 char:carbon mixture from a pure char mixture, but it can effectively distinguish a 1:1 mixture.

The carbon content of the char was found to have a strong influence on its behavior on the electric panel. Twenty grams of a regenerated char of 8.7% C content had a 99.3% separation efficiency (i.e., 99.3% of the char remained on the plate after pulsing the plate for 15 min.). By contrast, 20 grams of a regenerated char of 10.1% C content had only 83.7% efficiency; that is, 16.3% of this char was ejected from the panel.

Table 5.--Analysis by X-ray fluorescence of mixtures of absorbents separated on an electric panel

Sample	% (X-ray Fluor.)		Interpretation of Results
	P	Ca	
Canesorb	1.00	0.43	Carbon control
Bone Char	11.73	20.21	Char control
1:1 Ch/C	6.26	10.73	Mixture control; half of char
4:1 Ch/C	11.84	20.56	Mixture control; same as char
1:1 On panel	15.24	24.34	Char enriched fraction
1:1 Off panel	1.79	1.83	Carbon enriched fraction
4:1 On panel	12.65	20.56	Same as char control
4:1 Off panel	4.68	7.40	Carbon enriched; some char

The presence of dust or fines also affected panel operation. Canesorb powder, obtained by grinding in a mortar, was very sensitive to an applied voltage, rising rapidly as a cloud. Numerous fines were left on the plate, but it was not completely coated. Bone char powder was also quite active, with dust transported off the panel in both direction. The smaller char particles were more likely to pick up a charge and be transported off the panel, thus interfering with the separation.

In summary, the separation of char-carbon mixtures by Masuda panels is potentially promising since carbon is largely ejected from the panel and bone char remains on the panel. Separation of mixtures is complicated by two factors--particle size and char composition. Very small char particulates may behave like carbon and be transported. In addition, bone chars with higher C content will also be more like carbon in their behavior than char with lower C content in the electric field. Relative humidity is not an important factor although lower humidity gave slightly better transportation in most cases. Char may shield the carbon from the field, but this can be largely overcome by evenly distributing the sample in the center of the plate and gently disturbing it mechanically with a brush during the run. Any type of practical

application of these panels for this purpose would likely require the use of several passes since the separation is an enrichment process rather than a total one-pass separation. Panels with a larger surface area could be used to separate larger samples.

Separation by D.C. fields. The ability of a D.C. electric field to separate the adsorbents was also tested. Historically, use of D.C. methods is well established in the separation of minerals from their ores (Moore, 1973).

The simplest configuration of electrodes utilized in this study consisted of two oppositely charged flat aluminum plates about 20 cm x 4 cm, placed 1 cm apart. Sample was placed in the center of the bottom plate and a voltage applied until the sample was ejected from the surface of the plate. In a D.C. field, both char and carbon were ejected, but carbon came off at a lower voltage than did char. The voltage at which a particle was ejected was referred to as the "clearing voltage". Particle size and composition both partially determined the clearing voltage.

The effect of particle size is demonstrated in Figure 4, which shows the relationship of particle size to clearing voltage on sieved samples of bone char and carbon. For both adsorbents, larger particles required higher clearing voltages. Bone char demonstrated a steep, linear relationship between particle size and clearing voltage. Carbon showed a fairly constant clearing voltage of 3300 to 4300 V for most of the particle size range. Both very small (300 microns) and very large (1600 micron) carbon particles required slightly higher clearing voltages. A sieving prior to separation may be advantageous in this type of system.

Sample application was difficult with the plates: there was no way to feed the sample continuously, and a great deal of electrical bridging and sparking occurred because of the large upper electrode surface. Therefore, other configurations of electrodes were tested which would create a field of suitable shape and size to exploit the differences in electrical properties between bone char and carbon and allow continuous sample application. The basic pattern consisted of an upper stainless steel ball electrode suspended over an electrode shaped like a bowl with a hole in the center (Figure 5). Sample was continuously fed to the dispersing cone, allowing even contact of the sample with the ball electrode. Particles were charged with a constant voltage in the field between the electrodes. The less active particles (bone char) fell into the center hole and were trapped inside for easy recovery, while the more active particles (carbon) were ejected outside of the bowl. To reduce sparking and bridging of the current, the ball electrode was covered with a latex rubber sleeve. Sparking caused disruption of the separation by violently ejecting all particles.

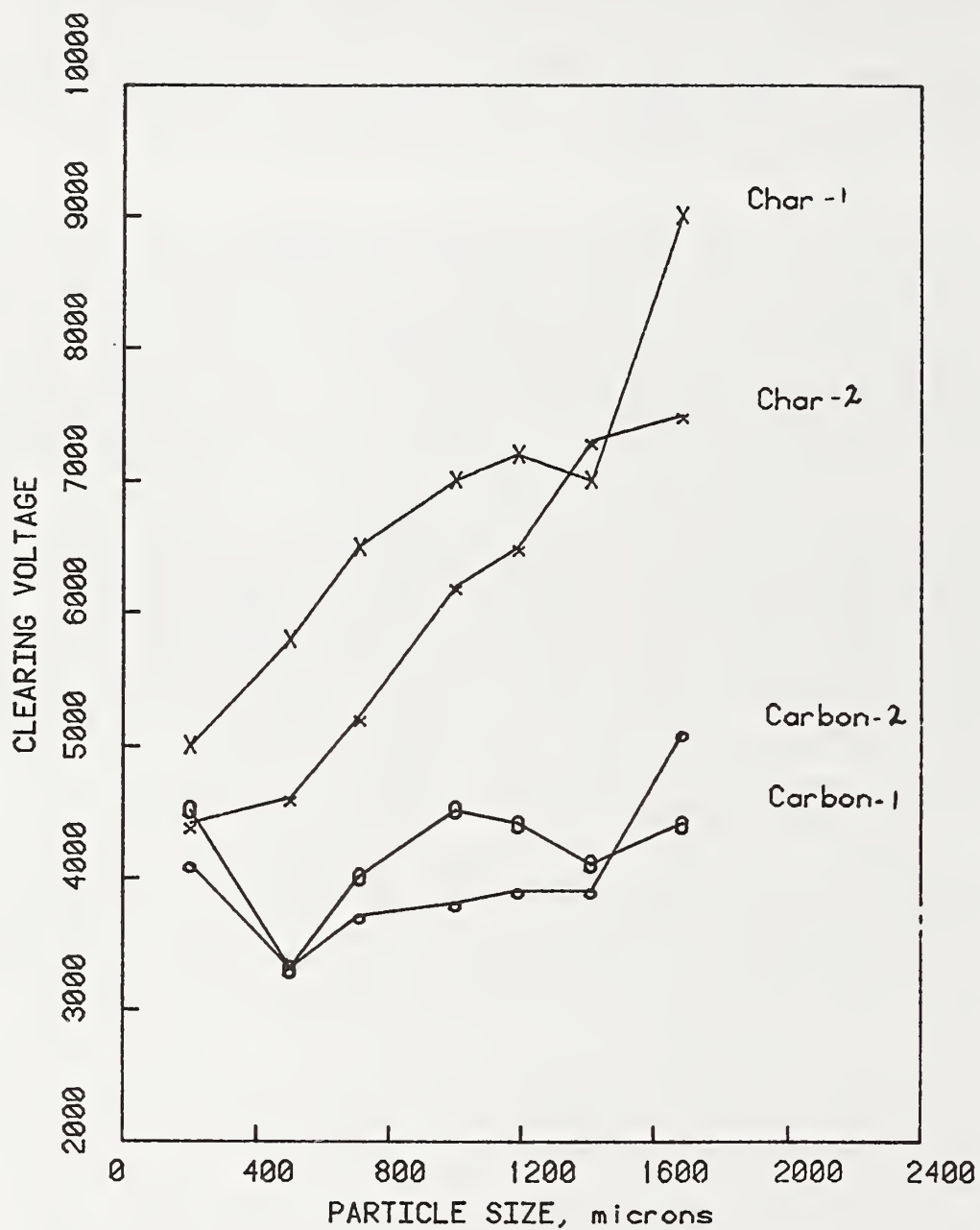


Figure 4.--Effect of adsorbent particle size on voltage required to eject particles from a D.C. field between charged aluminum plates. Conditions are described in text. The data represent two experiments.

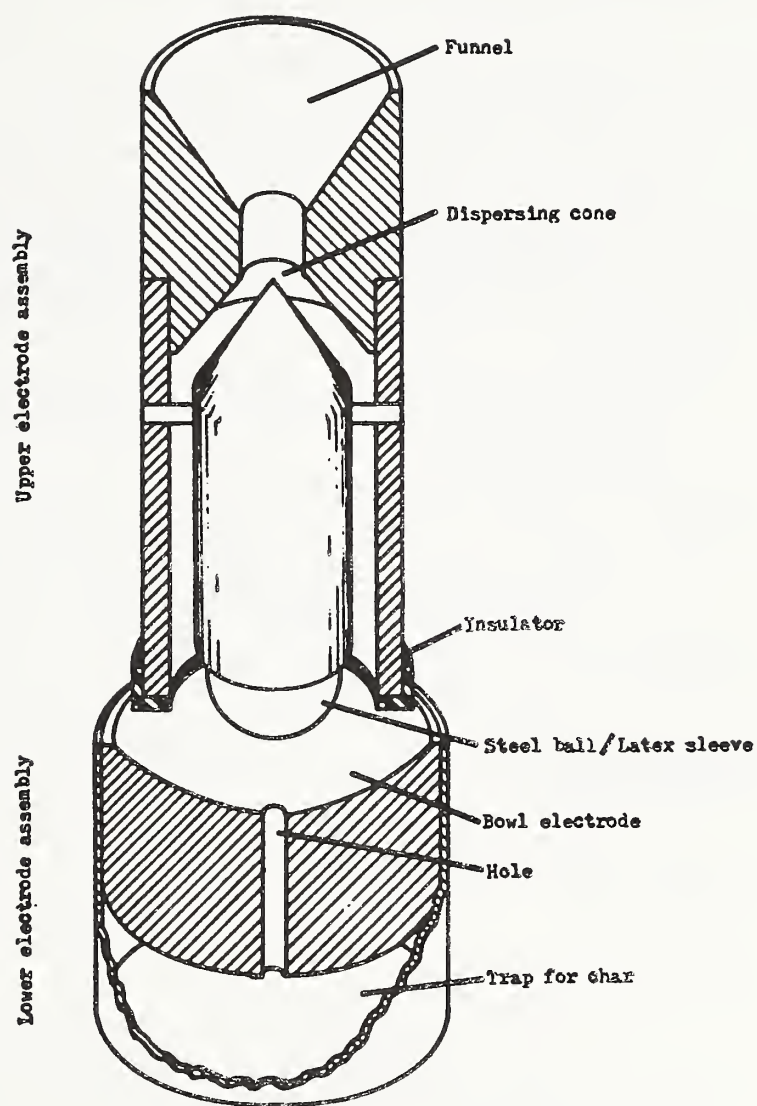


Figure 5.--Apparatus used in electrostatic separation of admixtures of bone char and carbon.

With this apparatus, 75 to 90% of the carbon was ejected and 50 to 95% of the char was trapped in the lower assembly. The amount of bone char that was trapped depended on particle size and voltage. The efficiency of several separations of individual adsorbents is summarized in Table 6.

Modification of the ball electrode to give a flat surface ("mushroom electrode") provided no real advantage in separation (Table 6). It was obvious, with the mushroom electrode, that a high voltage (5000 V) activated the bone char, causing it to be ejected along with the carbon, especially the smaller particles, but that a low voltage (3000 V) lessened the efficiency of carbon ejection out of the bowl, especially for particles larger than 1 mm. The ball electrode gave a better separation of bone char with some associated loss of ability to separate particles of carbon larger than 1 mm.

Table 6.--Effeciency of separation of seived fractions of absorbents in a direct current field in the ball and bowl separator

<u>Efficiency of Separation</u>			
Sample	6500V, B*	3000V, M	5000V, M
Carbon, <1mm	94	85	93
Carbon, >1mm	70	76	92
Char A, <1mm	54	43	34
Char A, >1mm	84	89	58
Char B, <1mm	80	32	17
Char B, >1mm	98	88	54

*B = ball shaped electrode; M = mushroom shaped electrode.
Efficiency of separation is expressed as a percentage of total separation.

SUMMARY

In summary, separation by electrostatic methods shows promise as a method to separate bone char from carbon. The absorbents exhibit markedly different behavior in both A.C. and D.C. electric fields, and it is possible to obtain fractions enriched in one or the other adsorbent by utilizing these differences. Particle size affects the separation, and a preliminary sieving is recommended.

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DISCUSSION

Richard Riffer, C & H: This is not really a question; it's a commentary on the subject. This electrostatic method looks very promising and may be the wave of the future. I think for the people who are now using the hydraulic method, I have an improvement that may be of interest. One reason the hydraulic separation doesn't work very well is that you have particle size contribution to the hydraulic properties as well as the density contribution. If you separate your mixture into two fractions, a coarse fraction and a fine fraction--take a sample of your mixture and run it through once to see where your halfway point is--and then run two hydraulic separations on the two fractions, you get a really nice, sharp separation. And furthermore, if you're using this in conjunction with a calcium analysis, the hydraulic separation doesn't significantly alter the calcium levels.

Godshall: How do you determine, Richard or anybody who's working with this, that you have a good, sharp separation? How do you determine that it's char vs. carbon?

Riffer: I think with some of the earlier hydraulic separations that we attempted, you get such a poor separation that you can't really see where you are. But if you get a sharp separation, it's easy to tell because the granular carbon is rather dull, whereas the char has a much glossier appearance.

Robert Kunin, Consultant: Isn't there a sufficient density difference between the two adsorbents to permit one to use a single float method with glycerin?

Riffer: We have studied solvent separation, but not glycerol specifically.

Godshall: As I mentioned earlier, there has been some work with solvents and density gradients, and I think the glycerin would be the same sort of idea. You can get a good separation, but then the adsorbent mixture is contaminated; and you can't really do any further tests on it. This is what you want to avoid.

Kunin: Why can't you rinse out the glycerin?

Frank G. Carpenter, U.S.D.A.: Bone char and carbon are powerful adsorbents. The glycerin would be chemisorbed and would not wash out.

Godshall: You would have to regenerate it. You can't get it all out by rinsing.

Kunin: What are the purposes for separating the char and carbon? Is the first purpose to separate and get a quantitative estimate of

percent carbon and char?

Godshall: That is one of the purposes. The other purpose is to do tests on the separated fractions, especially the bone char fraction to see how efficiently it has been regenerated. The question is what's happening with your bone char because you're pushing the system a little bit and one of the questions is, are you burning off too much of the carbon in bone char. You know, the actual elemental carbon content. You may ruin the bone char.

Kunin: Is separation without washing?

Godshall: The idea is to get it as unchanged as possible from how it comes out of the regeneration process without adding anything to it.

Riffer: In the plate configuration, did you say how long it takes to make a separation?

Godshall: Yes, that is very quick. It takes a few seconds. Let's say five grams can be separated in about thirty seconds. We were working with 1 gram samples. It depends on how much voltage you try--the higher the voltage, the quicker the separation. But you want to control your voltage.

COLOR TESTS AND OTHER INDICATORS OF RAW SUGAR REFINING CHARACTERISTICS

Margaret A. Clarke, Rebeca S. Blanco, and Mary An Godshall

Sugar Processing Research, Inc.

INTRODUCTION

The traditional indicators for raw sugar quality are polarization, color, invert, moisture, ash and grain size, most of which have been used as quality indicators in the market place for the last twenty five years. Other criteria, such as the ratio of invert level to ash level, or invert level to total non-sugar solids are used by some refineries. No single quality indicator or ratio is fail-safe; sugars of high pol may contain unusually high levels of starch and dextrans which drive up the pol, or a low color level sugar may be unexpectedly difficult to decolorize. A low quality sugar may have an ash to total non sugar ratio half that of a very high pol sugar. Quality indicators as a set are more meaningful than individual tests.

There have been considerable developments in research into sugar colorant, its composition, and the chemical nature of the colors, in recent years (Smith and Gregory, 1971; Kennedy and Smith, 1976; Carpenter, Roberts and Clarke, 1974; Smith, 1976). Knowledge about the different types of color and their interactions in refinery clarification and decolorization processes can explain the differing behaviors in processing of sugars of apparently similar color and quality factors.

The goal of the current study is to apply the knowledge about sugar colorants to compile a series of simple tests that will indicate the behavior of a raw sugar in any set of refinery processes. The tests must be rapid and uncomplicated. Test results need not be quantitative. Results are to indicate nature and degree of colorants present in the sugar, with regard to its processing efficiency, not to be analytically rigorous. A rapid estimate of the nature of colorants in a raw sugar should indicate if the sugar will decolorize well or poorly over bone char, granular carbon, or ion-exchange resin;

if color is the type to remain in the crystal, or to develop further in the pan and reduce the number of white boilings, and, perhaps, if color removal can be improved by a pH or temperature adjustment in process.

Nature of Sugar Colorant

A brief review of sugar colorant composition is appropriate at this point. Colorant may be considered as coming from two basic sources: the sugarcane plant and the process. Of the four general types of colorant, the phenolics and flavonoid class comes from the cane plant, where they exist as glycosides attached to sugar residues. The greatest research advances in recent years have been made on this class of colorant, first identified by the C.S.R.R.P.I. group, (Farber and Carpenter, 1971) and elucidated and expanded greatly by Dr. Peter Smith of CSR Ltd. (Kennedy and Smith, 1976; Smith and Gregory, 1971; Linecar, Paton and Smith, 1978; Paton and Smith, 1982) who has identified the major classes of phenolics and explained their roles in sugar production and refining. Smith found that approximately two thirds of the color in a raw sugar came from the phenolics and flavonoid group. Some phenolics are not colored as they come from the plant, but oxidize, complex, or otherwise react (sometimes with amines) to form colorant during process. Other plant colorants (anthocyanins, chlorophylls, carotenes) go into cane juice, but are generally removed in factory clarification, although Roberts has observed some chlorophyll-type compounds in raw sugars (Roberts, 1980). Plant colorants tend to be charged, more so at high pH, and, if unreacted, are of low to mid molecular weight range, where average size molecular weight is considered to be about 5000. Figure 1 shows the structure of the tricetin flavonoid group, a major color contributor.

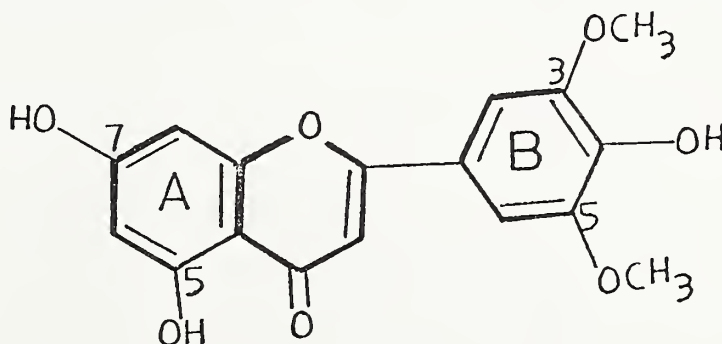


Figure 1.--Structure of the tricetin group of flavonoid pigments. Position 7 is the position of attachment of sugar residues in the naturally occurring forms of this compound.

Of the colorants developed in process ("factory colorants"- Smith and Gregory, 1971) caramels, melanoidins, and alkaline degradation products of fructose are the three subclassifications. Caramels are thermal degradation products of sucrose, only slightly charged, and of increasing molecular weight with increasing time and temperature and development. Melanoidins are, in general, Maillard reaction products of amine compounds with a sugar group. Since amine compounds come from the plant, this colorant is also plant-derived, but melanoidin colors do not form until the mixture of reactants is submitted to heat. Quite low heat over long periods, as under storage conditions, can develop this color. Melanoidins have some charged nature, usually negative at process pH's, but this tends to diminish with increasing molecular weight, and can be reversed by dropping the pH to the acid side. A sub-class of high molecular weight, uncharged compounds called melanins are particularly difficult to remove in processing. These high molecular weight colorants can be removed by factory clarification, but are preferentially occluded inside the crystal during crystallization.

The alkaline degradation products of fructose (ADF) are usually formed in the refinery, where pH is on the basic side. They are relatively uncharged and of medium to high molecular weight.

In addition to these four classes of colorant, there are several non-colored compounds that can develop color, or react to form color, in process, known as color precursors. These include amino acids and many hydroxy acids and aldehydes that are classed as simple phenolic compounds, iron, which complexes with phenolics to make colored compounds (Fleming and Parker, 1968) and reducing sugars. Potential color formation from these color precursors must be taken into account in considering color development in process.

The tests applied to raw sugars in this study, in order to gain as much information in as short a time and with as simple procedures as possible, include tests for phenolics, amino nitrogen compounds, alcohol precipitable material, and color at different pH values. This series of tests was developed by Dr. Peter Smith for use on cane juice (Smith and Gregory, 1971); we have made some modification and additions for use on raw sugar to correlate with refinability. We chose to use a phenolics test based on the molybdotungstate reaction rather than the sulfanilic acid test, and to use an 80% ethanol alcohol precipitate rather than a 50% methanol. We also included a test for iron (the bathophenanthroline spectrophotometric test) because of the importance of iron in color formation, but found that the information gained, over a wide range of sugars and refinery processes, did not

compensate for the time spent on the analysis. However, it is recommended that any refinery using ion exchange resins include a test for iron to this group, since iron can block resins badly.

The ratios of sugar colors at various pH values in whole and washed raws give several types of information. As the pH is increased, the different types of colorant react differently. Phenolic and flavonoid compounds have the greatest pH sensitivity, because of the molecular rearrangements that can occur under alkaline conditions to give a series of conjugated double bonds, the structural requirements for visible color. These, for example, are the rearrangements that occur in non-colored phenolics as they tie up with ferrous or ferric iron to form complexes that are colored. It is the phenolic compounds, the major contributors to raw sugar color, that are mainly responsible for the change in sugar color with pH, the change that makes sugar color impossible to measure reproducibly without constant pH. This change is most sharp in the pH 5.5 to 8.5 range, and levels off at higher and lower pH's. The pH7, although the official pH for the ICUMSA color measurement, is not appropriate for good reproducibility. Indicator value, or I.V., is the term applied to the ratio of color at pH 9 to that at pH 4, measured at 420 nm. The indicator values of pH insensitive colorants, that is, of melanoidins and caramels, are relatively low, because the molecules do not form significant color with increasing pH. A range of I.V.'s for the four types of colorant is shown in Table 1.

Table 1.--Range of Indicator Values (I.V.) for Sugar Colorants

Colorant type	I.V.
Melanoidin	1.0-1.2
Caramel	1.0-1.5
Alkaline degradation products of fructose	1.5-3.2
Phenolics and flavonoids	5-14

The alcohol precipitate test indicates two properties of the sugar: The total amount of polysaccharides present, which is shown by the volume of precipitate, and the amount of high molecular weight colorant, shown by the intensity of color of the precipitate. A precipitation with 80% alcohol gives a

more complete precipitation of potentially harmful polysaccharides than does 50%, and ethanol is more usually found nowadays in sugar laboratories than is methanol.

Total polysaccharides were also analyzed for this study, to provide a comparison with the alcohol precipitate test, but the total polysaccharides analysis is not proposed for inclusion in the series of color tests.

MATERIALS AND METHODS

Laboratory affination of sugars. The Hawaiian method was used (Meade and Chen, 1977). The Hawaiian method uses 66.0 refractometric Brix sugar syrups to wash 300 g of sample four times, each time using 450 ml of syrup and draining that out by vacuum. Then the sugar is washed with methyl alcohol and isopropyl alcohol and dried under vacuum.

Recommended test sequence. The alcohol precipitate test should be run first, and allowed to settle while other tests are run. The color tests should be started next, and time required for pH adjustment can be used to begin the phenolics and amino nitrogen tests.

Test solutions. 25 g of whole raw sugar was made up to 100 ml with distilled water in a volumetric flask. 25 g of washed raw sugar was similarly made up to 100 ml in a volumetric flask, with deionized water. These solutions were used for all subsequent tests.

Amino Nitrogen Test

1. Pipette into a test tube 1 ml of a 5% (w/v) sugar solution or 1 ml of cane juice (diluted 10:1). 10 mls of the 25% sample solution may be diluted to 50 ml to use for this test.
2. Add 1.5 ml acetate buffer solution, shake well.
3. Add 1.0 ml of 0.0002 M KCN reagent solution.
4. Add 0.2 ml of ninhydrin solution, shake well.
5. Cover test tubes (stopper loosely, with, for example, plastic insertion stoppers from volumetric flasks).
6. Heat in boiling water bath for 15 minutes. Do not allow evaporation.
7. Cool for 5 minutes.

8. Add 10 ml of 50% isopropanol-water, washing condensed vapors off stopper.
9. Read at 570 nm, within 15 minutes, against 50% propanol as reference.
10. Use 1 ml water to prepare blank. Subtract blank reading from sample absorbance.

Amino Nitrogen Test Reagents:

1. Acetate buffer, pH 5
100 ml of 20% sodium hydroxide
+200 ml of 20% acetic acid solution
2. KCN reagent solution
Prepare stock solution of potassium cyanide:
0.1628 g potassium cyanide in 250 ml water, to give 0.01 M KCN. Stock solution keeps for 3 months.

Dilute 5 ml of stock solution to 250 ml with methyl cellosolve. This reagent (KCN Reagent) is stable for 1 month only.
3. Ninhydrin solution
5 g ninhydrin in 100 ml methyl cellosolve. Keeps stable for 6 months in the dark. Store in brown bottle.
4. 50% isopropanol.
100 ml isopropanol
+100 ml water

Amino Nitrogen Standard Curve:

1. Glutamic acid, 0.1000 g in 100 ml distilled water.
2. 1 ml of this soln. = 95 μg amino nitrogen
3. Dilute aliquots of 1 to 10 ml to 100 ml each, to give standard solutions containing from 0.95 to 9.5 $\mu\text{g}/\text{ml}$ amino nitrogen.
4. Run test on aliquots of these solutions; construct standard curve of absorbance at 570 nm against $\mu\text{g}/\text{ml}$ amino nitrogen.

Phenolics Test

Of the 25% sample solutions, 5 mls was diluted to 50 ml in a volumetric flask with distilled water. The test may be used on cane juice diluted 10:1 with distilled water. To 2 ml of the dilute solution was added 0.2 ml Folin-Ciocalteu Reagent (Sigma Chemicals) and 0.4 ml of 2N NaOH. The reaction mixture was stirred for 5 minutes, and the absorbance read at 650 nm against a blank prepared with water and the reagents.

A calibration curve was prepared with caffeic acid, to give values from 1 to 12 µg/ml, as absorbance versus µg/ml.

Alcohol Precipitate Test

To 20 mls of the 25% sample solution in a 150 ml beaker is added 80 ml absolute ethanol. The beaker is covered and left to sit, preferably in the refrigerator, for 1 hour or more.

pH Measurements

The pH measurements were made at pH 7, 420 nm, according to ICUMSA Method 4 (after filtration through 0.45 µ filter). The pH's on these solutions were adjusted with HCl or NaOH for subsequent readings at pH 4 and 9. An Orion Model 501 (Orion Co., Cambridge, Mass.) pH meter was used, with a Corning Combination electrode.

Spectrophotometric Test for Iron

This procedure reduces all soluble iron to Fe(II) and determines this as a color complex. The test has the advantage that it can be used on raw sugars and dark colored samples.

Reagents:

Bathophenanthroline: 240 mg bathophenanthroline in 200 ml of 95% ethanol.

Hydroxylamine hydrochloride: 10 g hydroxylamine hydrochloride in 100 ml of 50% ethanol.

Chloroform

Buffer Solution: Prepare 0.2M acetic acid (12.0 g concentrated acetic acid made up to 1 liter with water) and 0.2 M sodium acetate (16.4 g sodium acetate dissolved in 1 liter water). Buffer solution is 14 ml 0.2M acetic acid + 9 ml 0.2M sodium acetate, made up to 100 ml with water.

Stock Iron Solution

173 mg $\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$ is dissolved in 50 ml water. Then 5.2 ml conc. H_2SO_4 is added, and the solution made up to 200 ml with water. This solution contains $100 \mu\text{g Fe}^{3+}/\text{ml}$.

Calibration Curve

For each standard solution, 25 g of very pure white sugar are dissolved in 50 ml water. Aliquots of 2, 5, 10, 20 and 30 ml of stock iron solution are added to these, and each is diluted to 100 ml with water, to give a series of solutions containing from $2 \mu\text{g/ml Fe}$ up to $30 \mu\text{g/ml Fe}$.

These standard solutions are used in the following procedure: Into a 125 ml separatory funnel, pipet 1 ml standard Fe solution, 1 ml hydroxylamine hydrochloride solution, 1 ml buffer solution, 12 ml water and 15 ml bathophenanthroline solution. Shake well and allow to sit 5 minutes.

Then add 10 ml chloroform and shake well, releasing pressure, then allow to sit for 5 minutes.

The chloroform layer (bottom) is drained off into a 25 ml volumetric flask and made up to volume with ethanol. The ethanol solutions are read at 533 nm, against a blank made with water instead of stock iron solution, and a standard calibration curve of absorbance vs. $\mu\text{g/ml Fe}$ constructed.

Sample Analysis

For raw sugars, a solution of 25 g sugar sample in 100 ml water is prepared.

Pipet 4 ml of this sugar solution into a separatory funnel, and add 1 ml hydroxylamine hydrochloride, 1 ml buffer, 9 ml water and 15 ml bathophenanthroline. Shake and let sit 5 minutes. Add 10 ml chloroform, shake well, release pressure, and let sit 5 minutes.

Drain the chloroform fraction into a 25 ml volumetric flask, and make to volume with ethanol. Read at 533 nm against a blank made with water instead of sugar solution. Read values off calibration curve, in $\mu\text{g/ml}$. For these quantities, the $\mu\text{g/ml}$ value is equivalent to ppm Fe in sugar.

For cane juice, the following quantities should be used:

- 5 ml cane juice
- 1 ml hydroxylamine hydrochloride
- 1 ml buffer

8 ml water
15 ml bathophenanthroline
10 ml chloroform

The calculation is:

$$\text{ppm Fe} = \mu\text{g/ml (from calibration curve)} \times \frac{1}{\text{g solids/100 ml}}$$

when g solids/100 ml is the concentration corresponding to Brix of cane juice.

Total Polysaccharides

The Roberts test (Roberts, 1982) for total polysaccharides, involving precipitation in 80% alcohol, filtration and subsequent phenol-sulfuric acid analysis was used.

Invert Sugars

Invert sugar was measured by gas chromatography as the trimethylsilyl ethers of the sugar-oximes on a Hewlett Packard Model 5880 Gas Chromatograph, using a fused silica capillary column, 12 m, coated with OV-101. The operating temperature was 175° C for 4 minutes, then programmed at 4° C/min. Methyl- β -glucoside was used as the internal standard.

Ash Measurements

Ash was measured using the ICUMSA conductivity ash method (Schneider, 1978).

RESULTS AND DISCUSSION

Results of each of the tests should be regarded as relative to one another, rather than as absolute values. Results must be taken in conjunction with one another to form a description of color of the sugar. One consequence of this work is that each refinery, or each set of processing operations, may find the type of sugar profile that it handles best.

The phenolics test gives an indication of the colorant that comes from the sugarcane plant. This type is usually negatively charged, especially at higher pH, and low to medium molecular weight. Some of this may have reacted further in process with amino compounds or aldehydes to form higher molecular weight material, with lower charge distribution. Phenolic colorant is best removed by processes that utilize the charged nature: clarification, especially Talo-type precipitation clarification, ion-exchange resin, and bone char. Both charge and molecular weight (solubility of

compounds) must be considered.

The amino nitrogen test gives an indication of the level of amine groups (usually from amino acids) in colorant. This test shows primary amine groups: many of the amine groups on colorant are secondary, some tertiary, but a suitable test for all is not yet available. The test used indicates how much reactive amine nature the colorant has, i.e. its potential to form high molecular weight uncharged colorant that is difficult to remove, and also indicates, to some extent, the level of Maillard reaction product colorant in the sugar. Sugars that have been stored for some time develop more color from reactions of amine compounds. Amine compounds can be removed to some extent by carbonatation, bone char, and granular carbon. Granular activated carbon is the only process effective in removing uncharged high molecular weight melanins.

The alcohol precipitate test precipitates all high molecular weight (over about 20,000) material in the sugar, and indicates both the amount of total polysaccharides and protein in the raw, and the amount of high molecular weight colorant. In general, the heavier the precipitate, the more total polysaccharide the sugar contains, and the darker color the precipitate, the more high molecular weight color it contains. Greyish colored precipitates are associated with high dextran levels, though this is not an infallible rule. Yellow or reddish brown colors tend to indicate high molecular weight phenolic colorant; grey-brown or duller colors are associated with uncharged high molecular weight melanoidin or melanin colorant.

The color values at different pH's give several types of information. The indicator value (I.V.) or ratio of color at pH 9 to pH 4, indicates the relative level of phenolic color: the higher the value, the greater degree of phenolic (charged, aromatic) material in the colorant.

The comparative I.V.'s of washed and whole crystal are particularly informative: If the I.V. is greater on the washed crystal than on the whole sugar, the percentage of phenolics is greater in the washed than on the whole raw, and, since phenolics are easier to remove than the other types of colorant, this is a good sign that the sugar will decolorize satisfactorily. Sugars with low I.V.'s in the washed crystal, particularly if the I.V. has decreased from the whole crystal to the washed, have high levels of amine color or caramel color, both of which are more difficult to remove than phenolic by most refinery processes. These sugars will tend to be more difficult to decolorize. Sugars with high levels of amine and caramel color often are those for which lower

grade white boilings have to be remelted. These are the sugars that cut back melt rate.

These ratios must be taken in conjunction with the absolute levels in the phenolics and amino nitrogen tests, and with the color of the alcohol precipitate to make an estimation of quality.

The sugars used in this study were from various countries of origin. AI and AII were from the same country, a major supplier to the U.S., and sugars AI, AII, B,C,D and G were all from Caribbean Basin areas.

Results of the phenolics tests and amino nitrogen tests are shown in Table 2.

Table 2.--Levels of phenolics and amino nitrogen compounds in whole and washed raw sugars

Sugar	Phenolics, ppm		Amino Nitrogen, ppm	
	Whole	Washed	Whole	Washed
AI	73	7	18	6
AII	96	12	34	8
B	70	10	19	5
C	116	2	37	8
D	88	5	18	5
E	85	1	20	9
F	92	1	14	12
G	48	1	9	4
H	85	15	72	6
I	92	4	11	5

Results of the alcohol precipitate tests are shown in Table 3, with total polysaccharide analyses alongside for purposes of comparison. The precipitate volume, or density, can be seen to give an indication of high or low level of total polysaccharides.

Total polysaccharide level in a raw sugar is important for several reasons. Most polysaccharides polarize high, and have a deleterious melassigenic effect. Several increase viscosity in process, adding to their melassigenic effect. They decrease filtration rates, and can physically block char, carbon or resin systems. If the polysaccharides go through, as they frequently do, to refined sugar, turbidity problems arise, and white sugars with high levels of polysaccharide will retain moisture and store poorly.

Table 3.--Alcohol precipitate and total polysaccharide levels

Sugar	Alcohol Whole	ppt Washed	Total Polysacch., ppm
AI	Med.-heavy grey-brown	Med.-heavy med. grey	886
AII	Med-light grey-brown	light greyish	728
B	Med-heavy grey-brown	v.Light pale yellow	1250
C	Heavy yellow-brown	med-heavy yellow-Brown	1260
D	Medium dk. brown	med-light grey-brown	785
E	Med-heavy dark brown	Med-heavy yellow-brown	1062
F	Heavy yellow-brown	medium grey-brown	1350
G	Med-heavy grey-brown	med-heavy dark brown	1591
H	Med-light yellow brown	very light pale yellow	Not determined
I	Voluminous dark brown	med-heavy dark brown	1400

Indicator values for the sugars are shown in Table 4. Included in Table 4 are washed crystal color as percent of whole color, measured at pH 7 and pH 8.5. These figures are included to show the pH effect on measurement of color distribution. Because phenolic plant-derived colorant, the relatively easily removed colorant, contributes in greater degree to color at pH 8.5, than at pH 7, sugars with a higher percent washed crystal color at pH 8.5 should tend to be comparatively easy to decolorize in a phosphatation-bone char, or carbonatation-bone char refinery. These are all only indications, not hard and fast rules, and all have exceptions.

Table 4.--Indicator values for whole and washed raw sugar

Sugar	Indicator Value		Washed Sugar Color as % Whole Color	
	Whole	Washed	pH 7	pH 8.5
AI	3.11	3.82	33	67
AII	4.13	5.02	21	21
B	2.55	3.41	16	19
C	1.75	2.91	12	14
D	3.11	3.88	37	27
E	1.86	2.61	23	22
F	2.55	3.73	13	12
G	2.27	3.02	33	40
H	2.11	4.33	17	29
I	1.55	2.20	12	13

Raw sugars submitted to these tests were all obtained from sponsoring refineries of S.P.R.I., and sugars shown in this paper were refined at plants using phosphatation and bone char.

In Table 5 are shown pol, along with the washed crystal color, at 420 nm, and the invert-ash ratio of the raw sugars, parameters often used as quality indicators. Refinery performance, as described by the refinery personnel, is summarized in this table.

Table 5.--Color, pol, invert/ash ratio and refinery performance of raw sugars

Sugar	Pol	Washed pH Color 420 nm	Invert/Ash Ratio		Refinery Performance
			Whole	Washed	
AI	96.87	1275	1.82	1.03	OK
AII	98.20	813	--	0.98	Good
B	97.41	714	1.37	0.74	Good
C	97.60	985	1.33	1.15	Blended, no problem
D	97.61	1081	2.07	1.53	Good
E	97.54	1159	2.73	2.38	Blended, no problem
F	96.88	985	2.03	1.37	OK
G	97.80	944	1.90	6.81	OK
H	98.57	521	0.6	0.75	V. good
I	97.54	1206	1.43	2.24	V. bad

Test Results and Decolorization Potential

A description of the type of color in each of the raw sugars studied, its probable behavior in the refinery, and any other observations readily apparent from the tests follows:

AI had fairly high phenolics and showed a lot of color in the crystal, but this is probably of the type that is fairly easy to remove. The polysaccharides were not especially high, so refining yield and losses should not be affected. The alcohol precipitate color showed some high molecular weight color remaining in crystal, which might be a problem, but this is probably phenolic (% color washed/whole). This should be an acceptable, good refining sugar.

AII had higher phenolics than AI, but had very little high molecular weight color in the crystal, and was low in polysaccharide, indicating low losses. This sugar should process well in phosphation-bone char, with no problems in decolorization.

Raw B showed above average phenolics but had very little color in crystal. It had fairly high polysaccharides, but most were in the syrup coating. This sugar should wash up well and process well.

Raw C showed a lot of high molecular weight colorant in crystal, which can be difficult to remove. This, plus rather high polysaccharides could cause processing difficulties and increase losses. This is probably an old sugar, stored for some time with subsequent increase in color development.

Raw sugar D had high color in the syrup coating, but should wash well. It had relatively low polysaccharides (somewhat unusual for a sugar of this type) and should process well, rather like AII, but perhaps not quite so easy to decolorize.

Sugar E showed very low indicator values, generally a poor indication for decolorization potential. A lot of melanoidin and high molecular weight colorant were apparent, much of it in the crystal. This sugar will probably be difficult to decolorize; color will stay in the crystal and lower the quality of white boilings.

Sugar F gave a color profile very much like that of Sugar B, with rather more amino nitrogen in the crystal. This sugar should refine well, but may have some hard-to-remove colorant. Polysaccharides are high, always a sign that losses may go up.

Sugar G displayed a heavy, dark-brown alcohol precipitate, indicating a lot of high molecular weight color. But the I.V.

of >3 and the brown rather than gray tone of the precipitate mean that this may be phenolic in nature, rather than the recalcitrant melanin type, and will decolorize readily.

Sugar H, a higher pol sugar, showed relatively high levels of phenolics in crystal, and a high indicator value. It had very little high molecular weight colorant in the crystal, and low polysaccharides, forecasting a good effect on yield. This sugar will not wash especially well, but should refine very well.

Sugar I showed a very low I.V. in the washed sugar--a bad sign. This sugar had a large amount of dark colored alcohol precipitate from the washed raw, which, taken with the I.V., indicates a lot of high molecular weight uncharged colorant in the crystal. The high polysaccharides will have a poor effect on yield. This sugar may be difficult to decolorize and process, and require considerable recycling.

The decolorizing predictions are summed up in Table 6, along with the refinery performance of the sugars. The general agreement of prediction with performance is good, with the exception of sugar E, which was predicted to be a problem sugar. Sugar E was processed only blended with other sugars, and had shown no problem under those circumstances. There was, probably fortunately, no opportunity to observe its decolorization behavior alone.

The information gained from this series of tests can be used to predict whether or not a sugar should be blended, or, if blending is not possible, if the refinery should expect trouble in decolorization and should adjust clarification parameters to improve liquor onto decolorization. Refineries employing ion exchange decolorization will find that sugars high in phenolic color will show increased decolorization if the liquor pH is increased (made more basic) so that as much charge is developed as possible. Some of the same improvement occurs with bone char.

Refineries using both bone char and granular activated carbon will find that incoming sugars with high levels of high molecular weight factory colorants (low charge) will decolorize better if given extra exposure to the carbon system.

Table 6.--Test predictions compared with refinery performance

Sugar	Prediction Based on Tests	Refinery Performance
AI	High crystal color but should be easily removed	No problem in refinery
AII	Should process well low losses	Ran very well
B	Should wash well, refine well	No problem decolorized well
C	Old sugar, high crystal color, may be problem	Blended; no individual report; no special problem observed
D	High color but should refine well	Refined well
E	Old sugar, lot of melanoidin in crystal, may lose lower boilings	Blended with A, no problem with recirculation
F	Possible crystal color hard to remove; poor yield	No special problem
G	High color but should process satisfactorily	No problem in color removal
H	Very low crystal color, should refine well; low losses	Excellent quality refined well
I	Old sugar, very bad color in crystal; difficult to process; high losses	Very poor to refine remelted 2 and 3 strikes, melt rate lowered 12%

Some advantages of this information can be seen in comparing Tables 5 and 6. The standard pol, color and invert-ash ratio measurements, although necessary and valuable, indicate that sugars D and E are quite similar, while D is actually a much better refining sugar. Table 5 data indicates that sugars AI and I may be much the same, with I the slightly better sugar. The reverse is true, as predicted by Table 6 data: Sugar I refined very poorly, while Sugar AI went through rather well.

This set of tests, which can be performed on a routine basis in about 1.5 hours, is, to reiterate, not an analytical, exact, accurate system. It provides a relatively rapid indication of decolorization behavior. On sugars reported here, the prediction was correct over 80% of the time; in additional tests performed in this study, not reported here, the 80% success rate is average.

SUMMARY

Color composition in cane sugars and their relationship to processing is briefly reviewed. A series of tests to determine relative amounts of color types in raw sugar is described.

Results of the tests on a series of raw sugars are presented and discussed, and their correlation with refinery performance (about 80%) considered.

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DISCUSSION

Ronald P. DeStefano, U.S. Sugar: This is more of an observation than a question, but maybe you can shed some light on it. You say pH 7 is not a good place to measure color?

Clarke: It's the pH of the official method, so it has to be used.

DeStefano: Is pH 8.5 much better? It seems to fall pretty much in the same part of the curve as pH 7.

Clarke: It's slightly better. The slope is not quite as steep at pH 8.5. The pH measurement is easier to make in that it will be more reproducible because the pH adjustment will be more likely to be accurate at pH 8.5 than it is at pH 7. But pH 9 is actually better because the curve of pH vs absorbance is flatter there.

DeStefano: The other thing is when you show the different classes of color, you said that as you go higher in pH you give a disproportionate weighting to phenolics. Since they tend to give you more color as you increase to pH 8.5 vs. pH 7, aren't you really going in the wrong direction to measure color as far as decolorization potential at the refinery? You said these phenolics are easy to remove compared to the other colorants, yet you are further overemphasizing their contribution to the sugar color.

Clarke: You're right. That's a problem. You can't go to a lower pH because if you drop the pH to where the slope is flatter on the acid side, you start to get inversion, and you can get color forming during the measurement.

Robert Kunin, Consultant: Do you measure the color of the alcohol precipitate visually in the beaker?

Clarke: Yes. This is a very simple visual procedure. When starting to use this procedure, it's necessary to precipitate several sugars to get some concept of the range of colors and volumes of precipitates. You can't just do one and make an estimate on that.

Kunin: Would it be easier to put it through a membrane filter and have discs to look at?

Clarke: That would not necessarily be easier. It would be more accurate, but would also be more complicated. We wanted to keep these tests simple.

APPLICATION OF GC ANALYSIS IN THE SOUTH AFRICAN SUGAR INDUSTRY

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INTRODUCTION

Several years ago the South African industry requested a specific and accurate method for measuring sucrose in cane juice and molasses with precision and repeatability at least as good as those of polarisation. The procedure would also need to be fairly rapid and be within the capabilities of factory analysts. If possible the analysis was to include fructose and glucose. This challenge led to an investigation of chromatographic techniques which clearly fulfilled the specificity demand. The next several years were spent in evaluating the accuracy and precision of such methods and the subsequent development of high throughput strategies.

At the initial implementation of this project (1972) it was felt that high performance liquid chromatographic (hplc) instrumentation was inadequate to the task e.g. resolution was poor, detection insensitive, temperature control inadequate, pumps pulsed and columns were both expensive and short-lived. Hence we concentrated on adapting, improving and developing suitable gas chromatographic (gc) techniques. Were the same brief to be issued today it is difficult to say whether we would persist with gc or persevere with hplc systems - the advances in hplc instrumentation and columns in recent years have certainly made the decision less clear-cut.

However, we were committed to gc and although initial results were promising they highlighted many

shortcomings such as tedious work up procedures, low precision (2-5% rsd) and inconsistent accuracy. Most of these factors were overcome and the improvements have formed the basis of a recent review (Schäffler and Morel du Boil 1984).

Once the accuracy and reliability of the technique had been adequately demonstrated the South African sugar industry was keen to apply the technique both in the area of factory control and for cane payment purposes. The first applications were usually in connection with particular research projects, but as wider acceptance was gained, these applications were extended to an industrial scale on a trial basis and subsequently adopted as official techniques to replace pol in some areas. This paper highlights a few of these applications.

PRACTICAL IMPLEMENTATION

Under present legislation the Sugar Industry Central Board (SICB) is responsible for the determination of the tonnages and analytical data relating to cane, final bagasse and mixed juice. Mixed juice and molasses analyses as well as the calculation of sugar recoveries and processing losses are based on sucrose measured using gc. The gc analyses of mixed juice have been under the control of the SICB in a centralised laboratory since 1979. Although earlier trials had indicated the feasibility of establishing separate gc stations at each factory, a single centralised facility was preferred. In 1982 the Sugar Milling Research Institute (SMRI) commenced gc analysis of final molasses for all factories. Periodic cross-checks between the two bodies are carried out and a formalised quality assurance programme is in operation (Schäffler and Day-Lewis 1983). This parallel monitoring forms the basis of the industry's analytical framework for payment and for factory control. The gc based figures have been recognised officially since 1982.

The analytical procedure is based on the analysis of a single weekly composite of mixed juice and of final molasses for each factory. Mixed juice is collected continuously over a period of one hour, preserved with mercuric chloride-potassium iodide and an aliquot (proportional to the hourly factory throughput) transferred to a polythene sachet (100 micron thickness) which is then heat-sealed and

immediately frozen at -40°C keeping the juice in a relatively thin layer. The samples are transported weekly to a centralised laboratory where they are thawed, composited and analysed for pol and brix and for sucrose, glucose and fructose. Pol and brix are compared with factory analyses, both as analytical control and to ensure that no degradation or dilution has occurred (Brokensha 1979). Typical data are presented in Table 1. Pol-to-sucrose ratios are calculated and applied to the pol tonnages for the week to convert these to sucrose.

Because of the centralisation of analyses on composite samples, results become available about one week later. Traditionally the local industry has used pol for day-to-day operational control of the factory and weekly composited results for factory balance and loss evaluation.

Inter-mill comparisons are of as much interest as week-to-week comparisons at a particular factory since a mill has to beat the industrial average to get a larger share of the available revenue. Many of the inter-mill differences in boiling house recoveries (BHR) and undetermined losses (UDL) have been shown to be due to inter-mill variations in pol-to-sucrose ratios in mixed juice and final molasses rather than to equipment or operational differences (Schäffler and Smith 1978).

IMPLICATIONS OF GC-BASED DATA

For a number of years the South African sugar industry used a direct polarisation procedure for the evaluation of cane quality and cane payment and for factory control. If there were a fixed relationship between pol and sucrose at all times and in all areas of process, this approach would have been acceptable. However, parallel monitoring of pol and sucrose confirmed that generally pol underestimated sucrose in all factory products and that this analytical bias was different at different factories and that there was also a strong seasonal effect.

Because of these differences, control parameters such as factory balances and recoveries based on pol were not necessarily comparable at different times of the year and inter-factory comparisons were questionable some of the time. These effects can be illustrated by way of examples extracted from data collected over the last three years.

Table 1.--Typical inter-laboratory analytical check
for mixed juice preservation

	*** POL PERCENT ***			*** BRIX PERCENT***		
	MILL	CLAB	ML-CL	MILL	CLAB	ML-CL
07/04/84	6.94	6.88	0.06+	8.96	8.99	0.03-
14/04/84	6.76	6.76	0.00+	8.72	8.76	0.04+
21/04/84	7.24	7.22	0.02+	9.22	9.24	0.02-
28/04/84	7.88	7.83	0.05+	9.97	9.96	0.01+
APRIL	7.37	7.34	0.03+	9.40	9.41	0.01-
TO DATE	7.37	7.34	0.03+	9.40	9.41	0.01-
05/05/84	8.26	8.21	0.05+	10.36	10.36	0.00+
12/05/84	8.42	8.37	0.05+	10.44	10.44	0.00+
19/05/84	9.09	9.06	0.03+	11.17	11.18	0.01-
26/05/84	8.91	8.90	0.01+	10.96	10.97	0.01-
02/06/84	9.09	9.06	0.03+	11.05	11.06	0.01-
MAY	8.80	8.77	0.03+	10.84	10.85	0.01-
TO DATE	8.52	8.49	0.03+	10.56	10.56	0.00+
09/06/84	9.51	9.50	0.01+	11.45	11.48	0.03-
16/06/84	9.50	9.48	0.02+	11.39	11.41	0.02-
23/06/84	9.57	9.53	0.04+	11.42	11.44	0.02-
30/06/84	9.72	9.70	0.02+	11.53	11.56	0.03-
JUNE	9.58	9.55	0.03+	11.45	11.47	0.02-
TO DATE	8.97	8.94	0.03+	10.94	10.95	0.01-
07/07/84	10.14	10.13	0.01+	11.97	12.01	0.04-
14/07/84	9.99	9.98	0.01+	11.77	11.78	0.01-
21/07/84	10.16	10.16	0.00+	11.94	11.97	0.03-
28/07/84	9.55	9.54	0.01+	11.20	11.21	0.01-
JULY	9.97	9.96	0.01+	11.73	11.75	0.02-
TO DATE	9.27	9.25	0.02+	11.18	11.19	0.01-
04/08/84	9.84	9.82	0.02+	11.49	11.47	0.02+
11/08/84	10.23	10.22	0.01+	11.89	11.87	0.02+
18/08/84	10.23	10.23	0.00+	11.85	11.85	0.00+
25/08/84	10.46	10.43	0.03+	12.14	12.12	0.02+
01/09/84	10.06	10.04	0.02+	11.64	11.63	0.01+
AUGUST	10.18	10.16	0.02+	11.82	11.80	0.02+
TO DATE	9.53	9.51	0.02+	11.36	11.37	0.01-

Boiling House Recovery

For a given amount of sucrose crystallised, this ratio is inversely dependent on the sucrose concentration in mixed juice and so any under-estimation in this analysis will tend to inflate BHR, giving a false impression of better performance than actually achieved - clearly the larger the underestimation the greater the misconception. For every 0.005 decrease in the pol-to-sucrose ratio the BHR is overestimated by about 0.44 units (Table 2).

Table 2.--BHR-seasonal effect of pol versus sucrose

	Pol/Sucrose in mixed juice	BHR based on pol	BHR based on sucrose
Factory A-May	0.975	88.6	86.4
Factory B-May	0.989	91.8	90.8
Factory A-Oct	0.995	85.6	85.2
Factory B-Oct	0.997	89.6	89.3
B vs A - May		3.2	4.4
B vs A - Oct		3.9	4.1
M vs O - A		3.0	1.2
M vs O - B		2.2	1.5

Using pol-based data, factory A performs better than factory B in May (3 units difference) and more so in October (4 units difference) - in fact the difference is about 4 units all season when compared on a true sucrose basis. The within factory decline in performance is about the same for both factories (1.2 to 1.5 units) on a sucrose basis but slightly worse for factory A (3.0 units) than for factory B (2.2 units) on a pol basis.

Factory Balances and Undetermined Loss

In general pol underestimates sucrose in both mixed juice and molasses. If polarising impurities were not affected by processing then it would not matter whether the factory balance were calculated using pol

or sucrose data. However, under South African conditions, there is always some positive pol generated in the factory so that the apparent sucrose in molasses will be inflated (although still less than the true sucrose) and undetermined losses will be underestimated. The difference varies from mill to mill and at different times at the same mill (Table 3).

It is worth noting that the true undetermined loss is about 20-60% higher than that indicated by the pol balance.

Table 3.--Undetermined losses - pol vs. sucrose

Mill	UDL(pol)	UDL(sucrose)	Difference
1	1.5	1.5	0
	1.7	2.6	0.9
	0.7	1.6	0.9
	1.8	2.9	1.1
2	1.1	1.4	0.3
	1.6	2.5	0.9
3	1.9	2.3	0.4
	1.8	3.0	1.2

Molasses Exhaustion

Both the quality and quantity of non-sucrose affect the exhaustibility of molasses. By obtaining reliable monosaccharide analyses on mixed juice it was hoped to be able to develop techniques of forecasting boiling house recoveries such that the juice quality influences could be divorced from the factory performance. To date these attempts have not been very promising.

However boiling performance is usually evaluated by means of an equilibrium molasses purity determined by correlation from the composition of various individual constituents. The difference between the calculated target purity and the true purity is a good indication of the degree of exhaustion obtained for a particular molasses. Because methods based on

the reducing properties of sugars tend to overestimate both the monosaccharides and sucrose, a target purity based on the more accurate gc determination of sucrose and monosaccharides has been developed. Although analytical anomalies have been eliminated, the formula probably still gives the factory with a low (F+G)/Ash ratio or low (F+G)/RS ratio a favourably high target for which to aim; (F = fructose, G = glucose, RS = reducing substance). The gc based formula tends to show less scatter and less seasonal trend than the earlier formula and is felt to be a better indicator of exhaustion performance (Ravnö and Lionnet 1982) (Table 4).

Table 4.--Difference between actual and target purities

Month	TPD(L.E.) ¹	TPD(GC)	Difference
1	3.0	5.2	2.2
2	1.2	4.6	2.4
3	2.1	4.4	2.3
4	0.6	4.7	4.1
5	1.2	4.4	3.2
6	1.5	4.4	2.9
7	1.9	4.9	3.0
(Max-Min)	2.4	0.8	

¹ TPD = difference between actual and target purity
L.E. = sucrose analysis by Lane & Eynon method.

Other Applications

GC analysis has been used in a number of less formalised applications at individual factories to monitor performance in different parts of the process. A recent investigation into sucrose balances (Purchase 1984) showed that a large proportion of the undetermined loss usually occurred between mixed juice and syrup, but that when high losses were experienced it was almost invariably in the boiling house (beyond the syrup stage) and was more prevalent towards the end of the crushing season.

An earlier investigation at a different factory, where the emphasis was on monitoring fructose and glucose, highlighted the fact that the change in fructose or glucose levels before syrup was relatively small, whereas monosaccharides were destroyed beyond the syrup stage - glucose to a far greater extent than fructose (Table 5) (Morel du Boil 1979). A possible link between high glucose losses and high sucrose losses has not yet been substantiated, although it has been shown in the laboratory that when large glucose losses occurred they were accompanied by some sucrose loss (Newell 1979). Purchase (1984) has also indicated that molasses sampled at periods of high factory loss, evolved gas and lost sucrose rapidly when heated in the laboratory.

Table 5.--Variation in fructose and glucose levels across process (Input level = 100)

	Fructose			Glucose		
	MJ ¹	S	FM	MJ	S	FM
Early season	100	80	86	100	85	75
Late season	100	95	90	100	97	57

¹ MJ = mixed juice; S = syrup; FM = final molasses.

Now that gc analysis has been officially adopted for cane payment purposes, the analysis of individual consignments has been proposed. Although this is not yet viable it has been shown that it is possible to analyse the low levels of monosaccharides in the cold digester extract by using either a splitless injection technique or a preliminary drying step (Morel du Boil and de Gaye 1983). Both methods are equally accurate, but the latter is more precise (Table 6). Sample preparation can be greatly simplified when sucrose is the only component to be analysed. The internal standard can be added volumetrically and direct silylation completed in about 1 minute. The gc analysis cycle can be shortened by using hydrogen as the carrier in short narrow-bore fused silica capillaries.

Table 6.--Comparison of split and splitless techniques (35 samples)

Sugar	Concentration range	Split mode		Splitless mode	
		Mean	s.d.	Mean	s.d.
Fructose	0.05-0.34	0.153	0.002	0.159	0.004
Glucose	0.05-0.30	0.153	0.002	0.154	0.004
Sucrose	3.2 -5.3	4.314	0.012	4.277	0.047

One of the often quoted drawbacks to sugar analysis by gc is that it is unsuitable for factory control. However, it has been shown that the gc procedure can be applied in a routine laboratory if required (Brokensha et al. 1978).

In extractions of low purity juices it has been shown that pol measurements are completely misleading with pol/sucrose ratios in the range 0.6 - 1.4 (Table 7) (Lionnet 1982).

Table 7.--Pol/sucrose ratio for low purity juices

Purity (pol/Bx)	Purity (sucrose/DS)	Pol/sucrose
9.5 + 6.1	15.9 + 3.7	0.66 + 0.44
15.7 + 3.2	27.3 + 1.9	0.61 + 0.10
17.7 + 4.6	11.8 + 5.6	1.37 + 0.60
32.1 + 13.6	38.2 + 12.4	0.86 + 0.15

Although ICUMSA no longer recognises the Luff-Schoorl procedure for reducing substances in raw sugars, the method is still being used to analyse South African export sugars. A recent comparison between the ICUMSA recommended Ofner method, the Luff-Schoorl procedure and a gc technique using splitless injection onto a bonded fused silica capillary column (SE-54) showed that the Ofner method consistently overestimated the monosaccharides and that this overestimation increased with decreasing sugar

purity. The agreement between the gc technique and the Luff-Schoorl method was acceptable (Dunsmore 1983) (Table 8).

Table 8.--Comparison of reducing substance analyses for raw sugars

Sugar Type	Reducing Substances			Differences	
	Ofner	Luff-Schoorl	gc	gc-O	gc-LS
VHP	0.21	0.18	0.19	-0.02	+0.01
SHP	0.32	0.27	0.26	-0.06	-0.01
HP	0.58	0.50	0.48	-0.10	-0.02
LP	1.12	1.02	0.97	-0.15	-0.05

The local fermentation industries now estimate yields based on gc analysis of molasses rather than chemical methods and report far more consistent agreement between predicted yields and those obtained in production.

DISCUSSION

In general, the industry is well satisfied that it has a technically and analytically superior method to pol and hence is more willing to evaluate cane quality and factory performance data critically. In most instances (e.g. BHR, UDL, target purity) the more accurate sucrose analysis has indicated that the real situation (i.e. in terms of sucrose) is generally worse than pol data lead one to believe. This in turn means that there is room for improvement. Inter-factory differences are now not as pronounced so that the other side of the coin indicates that most factories are performing at similar levels of efficiency.

A number of ancillary techniques developed in connection with the implementation of gc are having useful applications. For example, adequate freeze-preservation of juice samples allows many centralised (or specialised) analyses with good analytical control and analytical quality assurance has been formalised and should form part of any analytical control system. At present gc is serving

as a reference method for sucrose, fructose and glucose while the current acceptance of such instrumental techniques has paved the way for methods such as hplc to be more readily accepted by technologists.

In special investigations the reliability, accuracy and specificity of the sucrose and monosaccharide analyses have highlighted process areas where losses may be more prevalent and it is probably this area of detailed evaluation which will be better and further utilised in future.

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DISCUSSION

Pat A. Traci, Universal Foods: You said you preserve your samples by freezing. What did you do with your molasses samples to preserve them?

Morel du Boil: They are just kept cool. They are not frozen.

Traci: You don't have any trouble with degradation at all?

Morel du Boil: No, we don't.

Stephen A. Brooks, Barbados Sugar Research Institute: What internal standards do you use?

Morel du Boil: We use two. We use xylose for the monosaccharides and trehalose for the disaccharides, particularly in mixed juice because the levels are very different, and we prefer a sugar as the internal standard for derivatization reasons.

Brooks: Did you have any problems with the Luff-Schoorl correspondence?

Morel du Boil: How do you mean, correspondence?

Brooks: When you did the Luff-Schoorl method, did you also compare the Lane-Eynon method to obtain a correspondence between the Luff-Schoorl and the Ofner?

Morel du Boil: Now, we did not do Lane-Eynon. Ofner is the method ICUMSA now recommends for raw sugars. We only compared the Luff-Schoorl and the Ofner to the GC method.

Brooks: Did you also do any comparison with HPLC?

Morel du Boil: That is a difficult question to answer in a short time. Trevor Chorn will tell you more about it later on. At various times, there have been a few investigations, but we were hooked into a GC system. We had to make the decision and we couldn't consider LC and GC at the same time. It's working on a routine basis and is successful so we don't really have the incentive now to go off and look at LC.

Brooks: Some of the early work that you did in South Africa was also done on the Reductomat. Have you taken the opportunity of looking at the Reductomat again in light of the information obtained by GC?

Morel du Boil: No, we haven't.

CURRENT APPLICATIONS OF HPLC IN SUGAR ANALYSIS

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INTRODUCTION

In a continuing effort to develop reliable and specific methods for analysis of sugars and sugar related compounds, high pressure liquid chromatography techniques have been further investigated in our research laboratory. In general, HPLC offers speed, simplicity and versatility. This paper will focus on the HPLC methods developed for sucrose, glucose and fructose, other sugars and sugar derivatives such as difructose dianhydride, lactic acid and aconitic acid, uronic acids and degradation products of an artificial sweetener.

SUGARS

Polarization has been utilized for many years as the major analytical tool for factory control and sugarcane purchase. Pol measurement is very rapid and quite reproducible, but gives only one number which is the algebraic sum of all contributing components. Therefore, the analyses are always subjected to multiple interferences. In contrast, HPLC analysis can provide more specific information about each individual sugar in the sample and an estimation of levels of polysaccharides and other substances. It also gives indications of process efficiency not available from polarization.

Many papers have been published by our group and others on the use of HPLC on the analysis of sucrose, glucose and fructose in raw and refined sugars, cane juice, process liquor and molasses (Clarke et al. 1977, Clarke & Brannan 1978 a, 1978 b, 1978 c, Clarke and Brannan 1980, Clarke and Tsang 1983, Wong-Chong and Martin 1980, Charles 1981). In general, direct HPLC analyses work well for cane juice (Wong-Chong and Martin 1980), molasses (Clarke and Brannan 1978, Rajakyla and Paloposki 1983) and low grade syrups (Clarke and Tsang 1983) where there are significant amounts of glucose and fructose.

For high purity liquors, the glucose and fructose peaks are overwhelmed by the sucrose peak, and additional post-column derivatization reactions may be required for invert determination (Lawson and Russell 1980, Wight and Niekerk 1983, Wnukowski 1983).

HPLC is routinely used in quality control in production of high fructose corn syrups and other corn products (Fitt et al 1980; Brobst and Scobell, 1981), and for process control by many sugar using industries such as canners, fruit juice manufacturers and soft drink manufacturers (David and Hartford, 1979; Richmond et al. 1981; Wade and Morris, 1982).

In all direct sugar analysis, detection is accomplished by differential refractometer. Early work by our group (Clarke et. al. 1977, Clarke and Brannan 1978a) and others (Meager and Furst 1975, Linden and Lawhead 1975) used amino bonded silica column with an acetonitrile-water solvent for carbohydrate analysis. Under these chromatographic conditions, the resolution of monosaccharides is generally poor. Other problems associated with carbohydrate determination using silica columns include the use of expensive and hazardous solvent (over 75% acetonitrile) and comparatively short column life as a result of the poisoning of the chemically bonded amino-phase packing material by the non-sugar coextractives, and the consumption of the reactive amine groups by Maillard reactions. The use of in situ modification of silica with amine, however, produces columns displaying greater long-term stability (Wheals and White 1979, Hendrix et. al. 1981).

It was not until the advent of ion-exchange type columns (Palmer and Brandes 1974) that HPLC became a really practical tool for sugar analysis in many products. Using only water as eluent, this kind of column is more durable i.e. has longer column life, and is simple to regenerate. Carbohydrate analysis using ion exchange type columns in the calcium form and refractive index detection has been found to be a useful method for process control in sugar factories and refineries (Clarke and Tsang 1983). This is the system used to control corn wet milling plants. Figure 1 shows typical chromatograms of molasses and cane juice.



Figure 1.--Typical chromatograms of molasses and cane juice.

Field Studies

The ion-exchange HPLC column technique is further applied in the studies of chemical plant growth regulators, cane deterioration and frozen cane conditions. In a comparative study of several commercial plant growth regulators done by the U.S. Sugarcane Field Station, Houma, LA (Legendre et. al., 1984), Polado showed consistently better activity than Fusilade for all varieties and at all sampling dates. In a series of tests on cane deterioration as affected by variety, also conducted at the U.S. Sugarcane Field Station, the variety CP 70-321 appeared less affected by the length of storage time after harvest, i.e. length of time the cane was left to lie in the field before milling, than other varieties studied. A study on frozen cane, reported at this SPRI Conference by Dr. Legendre, showed not only the expected decrease in sucrose content and increase in fructose and dextran content with time after freeze but also a sharp difference in sugar levels and dextran formation among different varieties. Sucrose analyses by HPLC were in good agreement with other factors analyzed in this study. These studies were all cooperative work between the U.S. Sugarcane Field Station, U.S. Department of Agriculture, Houma, Louisiana, where the field studies were conducted, and

S.P.R.I., where the analytical work was done.

Organic Acids

Separation of lactic acid and trans-aconitic acid from sugars can be accomplished on a HPX-87H cation-exchange column, containing sulfonated polystyrene-divinylbenzene copolymer, with diluted sulfuric acid as the eluent (Tsang and Clarke, 1983). Using refractometric detection, trans-aconitic acid elutes after fructose, followed by lactic acid (Figure 2). Low levels of aconitic acid in cane juice can be detected with either a 215 nm or 254 nm UV detector, but lactic acid can only be measured at 215 nm. Higher levels of these acids can be detected by refractive index. Lactic acid is a byproduct of bacterial action on sugars and its presence will interfere with the results of analysis of reducing sugars because it reduces copper in the Lane-Eynon procedure.

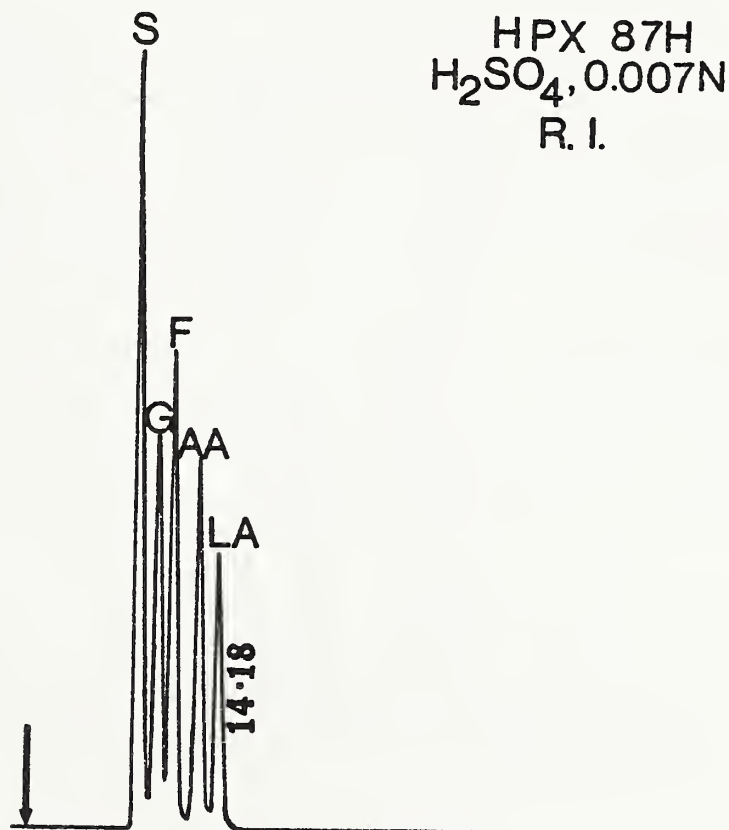


Figure 2.--Separation of lactic acid and aconitic acid.

Uronic Acids

We have found that uronic acids can be analyzed on an HPX-87H ion exchange column system. This system can be used to differentiate between galacturonic acid and glucuronic acid. Uronic acids are associated with cell wall and other plant polysaccharides, and are very difficult to separate and analyze. Glucuronic acid has been found in indigenous sugarcane polysaccharide (Roberts and Godshall, 1978) but no galacturonic acid (pectin component) has been observed in sugarcane. The presence of a certain uronic acid can give an indication of the type of polysaccharide present. Glucuronic acid elutes just before galacturonic acid on the Bio-Rad HPX-87H column with detection by UV 215 nm. Glucuronic acid and its methyl derivative had been identified in the gum arabic methanolyzate (Figure 3). Table 1 shows conditions of analysis for both organic acids and uronic acids.

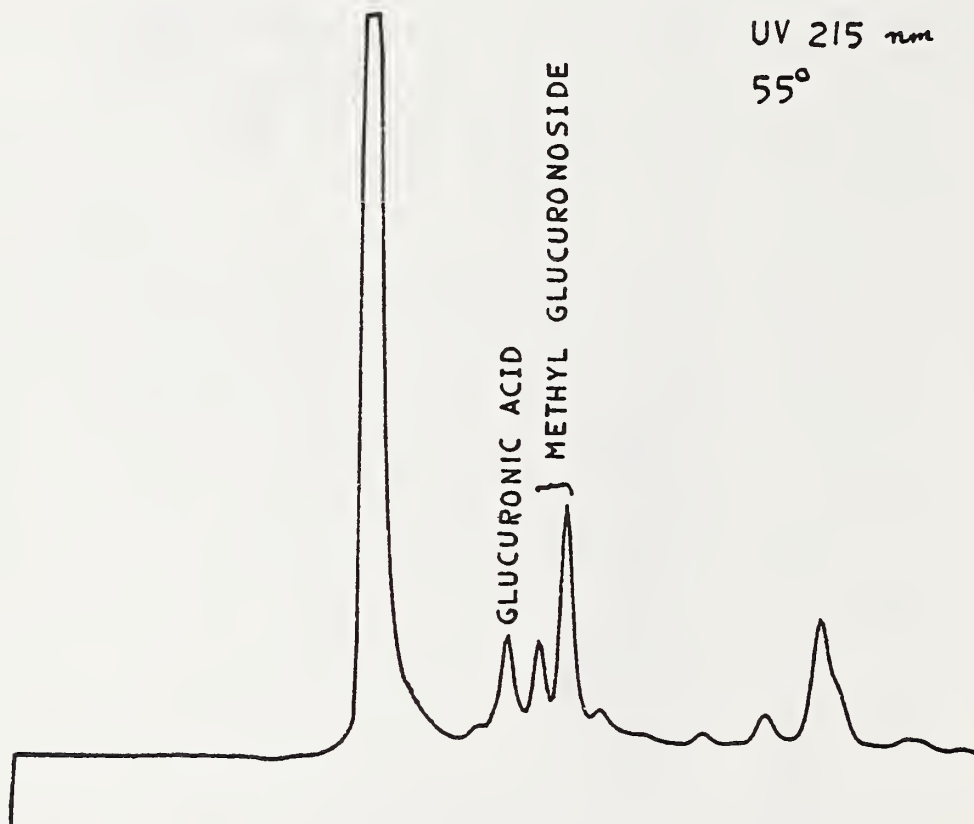


Figure 3.--Determination of uronic acid residues in methanolyzate of gum arabic.

Table 1.--HPLC conditions for organic acids and uronic acids

	Organic Acid	Uronic Acid
Column:	Aminex HPX-87H	Aminex HPX-87H
Solvent:	0.007N H ₂ SO ₄	0.007N H ₂ SO ₄
Column Temperature:	Room Temp.	55° C
Detector:	Differential refractometer UV 254 (Aconitic acid) UV 215	UV 215

Polysaccharide Hydrolysis Products Identification

An important technique in polysaccharide identification is hydrolysis, usually acid, of the polysaccharide into its component sugars and subsequent identification of these components, usually by some chromatographic procedure. HPLC can give both qualitative identification and quantitative estimation of the component sugars.

Certain monosaccharides are difficult to separate even on the calcium ion-exchange systems that give maximum resolution. This problem may be overcome by the use of ion-exchange polymers in the heavy metal form as solid phase. Using a BioRad HPX-87P carbohydrate analysis column, wood sugars in samples from the pulp and paper industry can be separated under isocratic conditions (Wentz et. al. 1982).

Current work has successfully separated component sugars from several sugarcane polysaccharides. Glucose, xylose, galactose, arabinose and mannose, all component sugars obtained from acid hydrolysis of polysaccharides, can be separated in less than 20 minutes as shown in Figure 4.

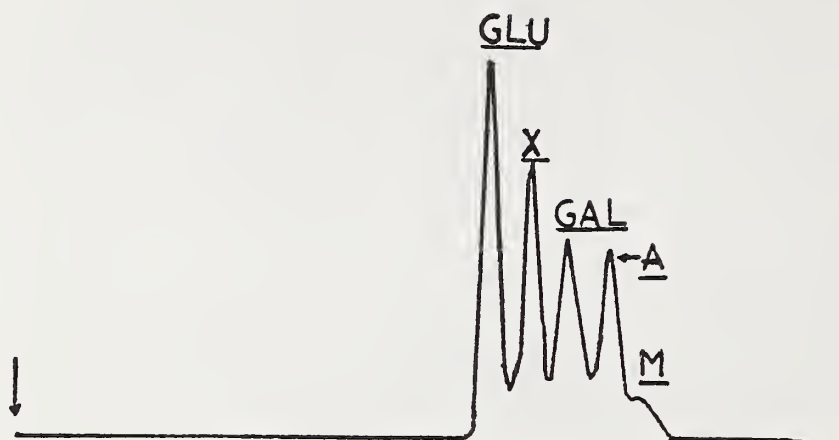


Figure 4.--Monosaccharides determination with ion exchange column in the lead (Pb^{+2}) form.

Difructose Dianhydride

Difructose dianhydrides have been reported as breakdown products of fructose in syrups and beverages. We have worked out HPLC methods to analyze the difructose dianhydrides rapidly. Pure dianhydrides 1 and 2 are prepared from acid-catalyzed dehydration of D-fructose (Wolfson et. al. 1952). The HPLC results, shown in Table 2, show that sucrose, 1, 2, and fructose can be separated on a silica column on a radial compression system modified in situ with TEPA, using acetonitrile and water (75:25) as the solvent, and detected by differential refractometer (Figure 5). Separation of these sugars can also be achieved in an Aminex HPX-87C carbohydrate column.

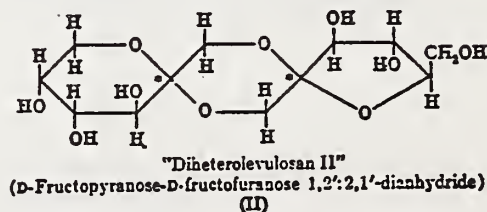
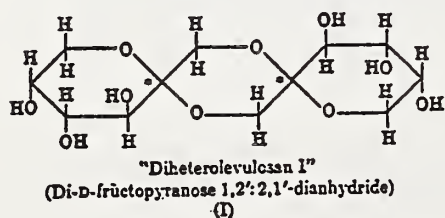
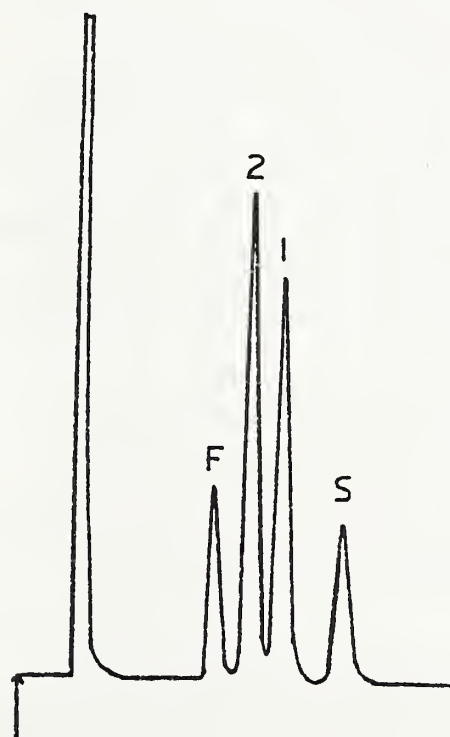


Table 2.--Separation of difructose dianhydrides from sucrose and reducing sugars on two HPLC systems

	Biorad Carbohydrate Column (H ₂ O)	Waters Silica Column
Structure	R.T	R.T
Sucrose	8.40	17.32
Difructopyranose 1,2':2,1' Dianhydride (1)	10.80	14.05
Fructopyranose-Fructofuranose 1,2': 2,1'-Dianhydride (2)	9.60	12.36
Glucose	10.20	12.36
Fructose	12.40	10.44



CONDITIONS

COLUMN - SILICA

MOBILE PHASE- CH₃CN : H₂O
(75 : 25)
0.01% TEPA

DET- R.I.

FLOW RATE- 1ml/min

Figure 5.--Separation of difructose dianhydrides on the silica column modified with TEPA, in the radial compression (RCSS) system.

Aspartame Deterioration

Aspartame, a low-calorie, nutritive sweetener, is currently used in soft drinks as well as powdered drinks and cake mixes in the United States. Aspartame is the methyl ester of the dipeptide aspartylphenylalanine and under a wide range of stressful conditions may be susceptible to degradation.

Since aspartame has been introduced into the market for only a short time, its stability in various food products has not been investigated fully. The stability of the artificial sweetener in various media was studied recently by Homler (1984). This author claimed that aspartame-based colas, stored at 20° C for 40 weeks, remained acceptably sweet relative to the saccharin-sweetened beverages. The levels of aspartame left in the carbonated beverages and its breakdown products, however, were not reported.

In a joint effort with the Sugar Association, Inc., we investigated the effects of aging on aspartame in soft drinks which had been stored at room temperature for an extended period of time and determined the identities of the degradation products. Four different brands of aspartame-based soft drinks, made and purchased in Canada, where aspartame has been in use for some years, were analyzed for aspartame and its breakdown products after various storage periods. The samples were analyzed by HPLC using a Waters μ -Bondapak C18 reverse phase column (3.9 mm x 30 cm) and a UV detector at 215 nm. The solvent used was acetonitrile and phosphate buffer (10:90) at 0.8 ml/min. The phosphate buffer was 0.17% monobasic potassium phosphate adjusted to pH 3.5 with phosphoric acid. This system was derived from a system recommended by the Alltech Associates, Deerfield, Illinois.

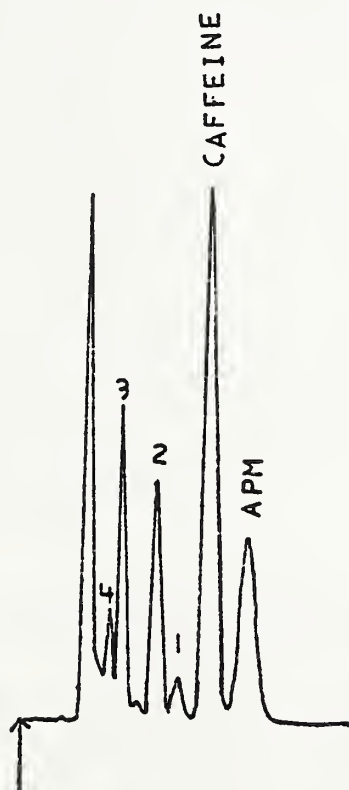
The results of the analyses are presented in Table 3. Clearly, aspartame is unstable in the beverages for any extended period of time, at room temperature. All samples except one contain less than 40% of the label claim for aspartame content after 6 months. Three of the four samples contain less than 10% of the original level of aspartame in 36 months. In only 4 weeks, over 10% of the aspartame has decomposed in most samples.

This study allows us to trace the development of various reaction product over a period of time. The degradation pattern of aspartame in various type of carbonated beverages appear to be very similar. Four breakdown products are identified by HPLC (Figures 6 and 7) and their relative proportions determined (Table 3). No aspartame condensation product has been identified in this study.

Table 3.--Aspartame levels in soft drinks stored over months

	Lime-Lemon #1	Lime-Lemon #2	Diet Cola #1	Diet Cola #2
Freshly Prepared	100%	100%	100%	100%
1 Month	89.7%	87.3%	94%	84.5%
6 Months	56.9%	36.9%	38.2%	28.2%
36 Months	13.72%	6.40%	4.10%	3.58%

6 MONTHS



36 MONTHS

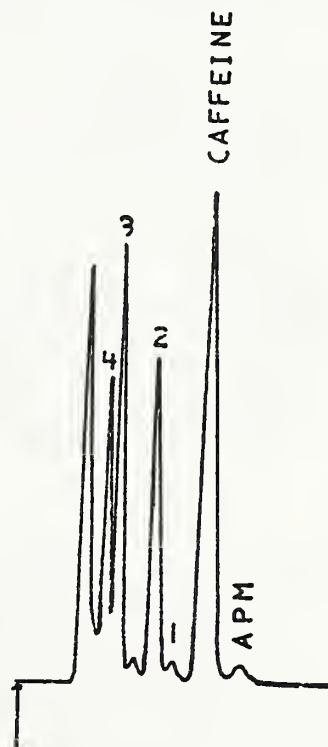
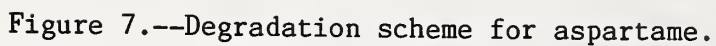


Figure 6.--Determination of aspartame (APM) breakdown in diet cola from Canada by HPLC.



SUMMARY

HPLC methods have been developed and applied to many analytical requirements in sugar processing research. Several of the application and analysis systems are described herein.

HPLC analysis using ion exchange columns in the calcium form and water as solvent has been found to be a useful method for analysis of sugar process syrups and juices. Sucrose, glucose and fructose can be analyzed individually and rapidly in a simple determination, thereby eliminating the need for multiple tests to conduct a complete analysis of the products. The technique has also been applied in the study of the effect of chemical growth regulators on canes, cane deterioration due to delayed milling, and deterioration in cane following a freeze.

HPLC offers the ability for rapid analysis of organic acids, in particular aconitic and lactic; this procedure eliminates lengthy classical methods for individual acids.

An HPLC method for analysis of difructose dianhydrides has been developed. Separation of two dianhydrides from sucrose and fructose can be achieved in 15 minutes. The technique provides an excellent monitor for the synthesis procedure of difructose dianhydrides.

Aspartame and its breakdown products in soft drinks have been measured with HPLC techniques, and the degree of breakdown measured over time. Several breakdown products have been identified and monitored over time.

The difficult separation and identification of uronic acid residues in polysaccharides has been accomplished, using HPLC identification of the acids. Identification of sugar moieties comprising polysaccharides has been simplified and quantified with HPLC methods, providing a useful tool in polysaccharide analysis.

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DISCUSSION

Stephen A. Brooks, Barbados Sugar Research Institute: Did you notice any large variations between the different types of columns that you were using to analyze the same substance?

Tsang: Do you mean the sugars? We have tried the amine columns, the Sugar-Pak column made by Waters, and also the Bio-Rad ion exchange column. We found that the Bio-Rad column was by far the most durable. After at least 2000 injections, it is still working beautifully, so we decided to stay with that type of column.

Brooks: The question is, in looking at the analyses that you report, were the results that you got with the Waters column comparable to the ones you got with the Bio-Rad column?

Tsang: We have not done that type of study. We have found that the Bio-Rad column gives much better separation and better reproducibility than the Sugar-Pak.

Randolph R. Tamaye, HSPA: I notice in your chromatograms that you are getting a lot of baseline separation, particularly the one where you showed the deteriorated sample of sugar with the dextran peak. What kind of sample clean-up did you have to do?

Tsang: For cane juice, we dilute the juice ten times and filter it on a 0.45 μ Millipore filter. We find that this is adequate, and we don't have to use the Sep-Paks for additional cleanup.

A COMPARISON OF GLC AND HPLC FOR THE DETERMINATION OF SUGARS IN FINAL MOLASSES

T. A. Chorn and A. Hugo

Tongaat-Hulett Sugar Ltd., Research and Development

INTRODUCTION

The South African Sugar Industry uses gas-liquid chromatography (GLC) to determine sucrose, glucose and fructose for cane payment purposes and also for the analysis of various factory samples. This technique has been well evaluated, validated and documented (Schaffler 1976, Brokensha et al. 1978, Schaffler and Loker 1974, Nurok and Reardon 1975, Kort et al. 1975, Schaffler and Day-Lewis 1983, Schaffler and Morel du Boil 1984, Schaffler and Morel du Boil 1981) and has given precise and accurate results. Recently high pressure liquid chromatography (HPLC) has emerged as a viable alternative analysis technique (Dutton 1982, Fitt 1978, Hugo 1984, Ivie 1982, Ivin 1980, Ivin 1983, Iverson 1981, Kort 1975). Although both techniques have been extensively described, very little has been published on the comparison of these two techniques for the analysis of sugars (Iverson and Buena 1981, Dutton 1982). As an analysis by HPLC is far quicker and less technically demanding than by GLC, these two techniques were compared to determine whether similar results could be achieved.

INITIAL EVALUATION OF HPLC

As early as 1982 (Chorn) we evaluated the Waters Sugar-analyser 1 for the analysis of sucrose in mixed juice and final molasses. The results obtained by HPLC are compared with the corresponding GLC data in Table 1.

These results showed, with the exception of one mixed juice sample, that the two techniques did not give comparable results. HPLC chromatograms of a sucrose standard, a molasses sample and a mixed juice sample are shown in Figure 1 (a), (b) and (c) respectively. In Figure 1 (b) it is evident that the sucrose peak is not well resolved from the peaks due to polysaccharides and inorganic salts. This inadequate resolution is often the cause of incorrect

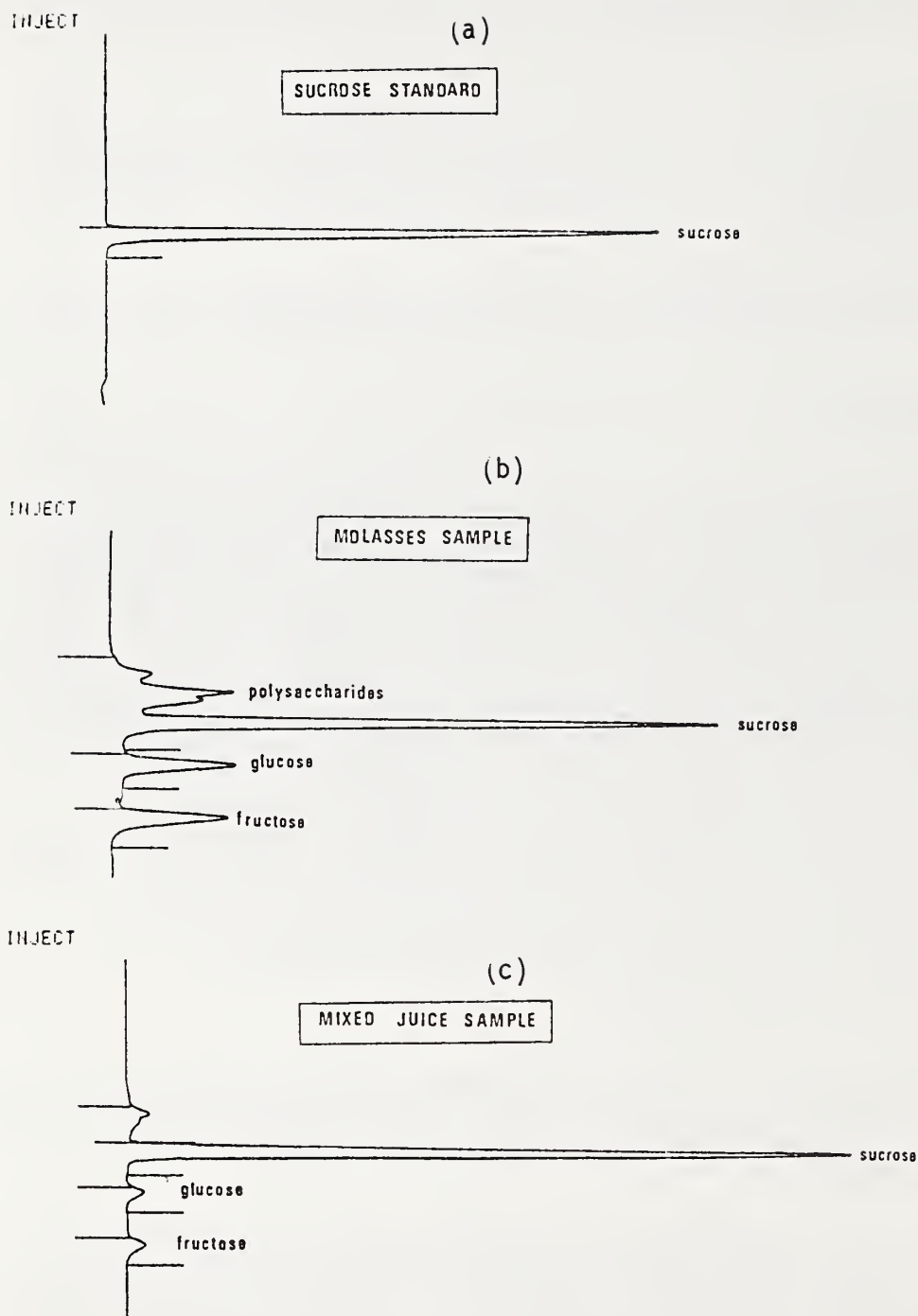


Figure 1.--Chromatograms of (a) a sucrose standard, (b) a molasses sample and (c) a mixed juice sample.

quantitation in any type of chromatography no matter how sophisticated the integrator may be. Therefore, it is of paramount importance in any quantitative chromatographic technique, where a high degree of accuracy is required, to ensure that the peaks of interest are completely resolved.

Gas-liquid chromatography of sugars is well advanced and uses capillary columns of a very high resolving power. A typical chromatogram of a final molasses sample is depicted in Figure 2 showing good resolution of the major sugars and partial resolution of minor sugars such as psicose, mannose and mannitol.

Table 1.--Comparison of HPLC and GLC for the analysis of sucrose in cane mixed juice and cane final molasses

Sample	Sucrose % (m/m) by		HPLC-GLC
	HPLC	GLC	
Mixed juice 1	12.02	10.65	1.37
Mixed juice 2	12.33	11.72	0.61
Mixed juice 3	10.80	10.08	0.72
Mixed juice 4	10.83	10.83	0.00
Mixed juice 5	11.44	11.15	0.29
Mixed juice 6	11.55	10.25	1.30
Final molasses 7	34.9	30.3	4.6
Final molasses 8	35.5	31.3	4.2

REASONS FOR POOR COMPARISONS BETWEEN GLC AND HPLC

1. Resolution

Since HPLC overestimates the sucrose concentration when compared to GLC, it is reasonable to assume that the HPLC technique includes other components with the sucrose peak.

An obvious way of increasing resolution is to transform the components of interest in a precolumn derivatisation reaction, but this leads to greater complexity of the method.

2. Influence of inorganic salts and acids

Although inorganic salts are well resolved from sucrose, it has been shown in this laboratory (Hugo 1984) that they can influence sucrose quantitation. The full reasons for this are not known but they may be ascribed, at least in part, to the change in the ionic environment of the analytical column as the inorganic salts elute. We therefore found it essential to remove these inorganic salts.

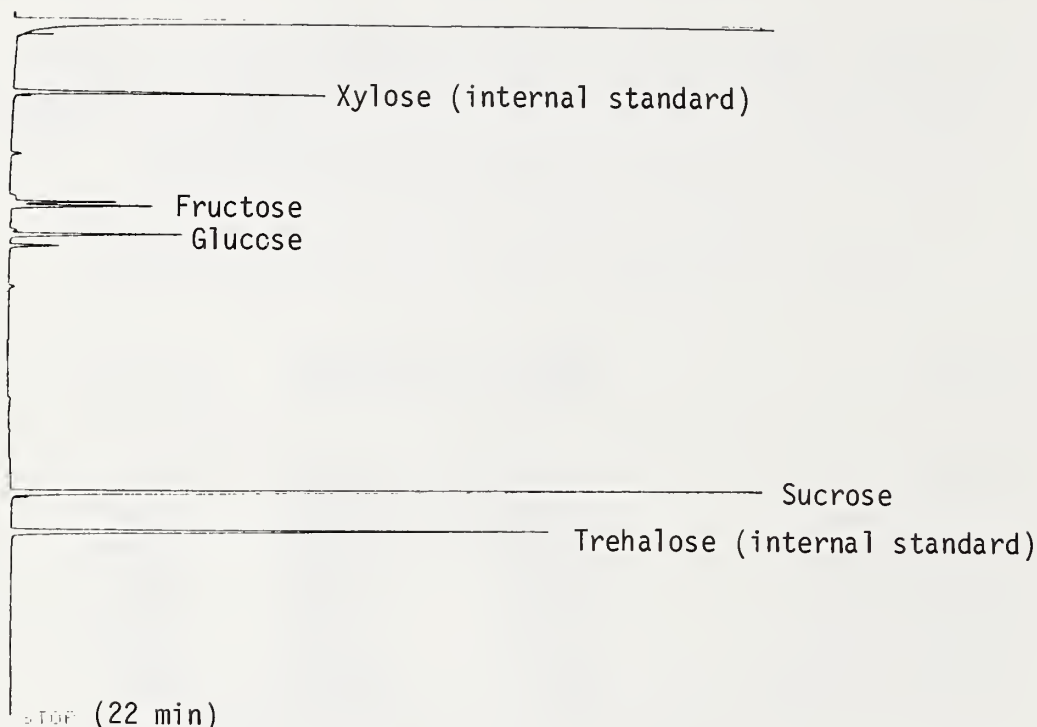
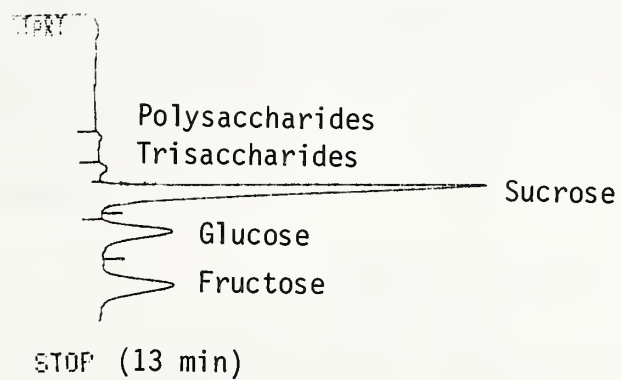
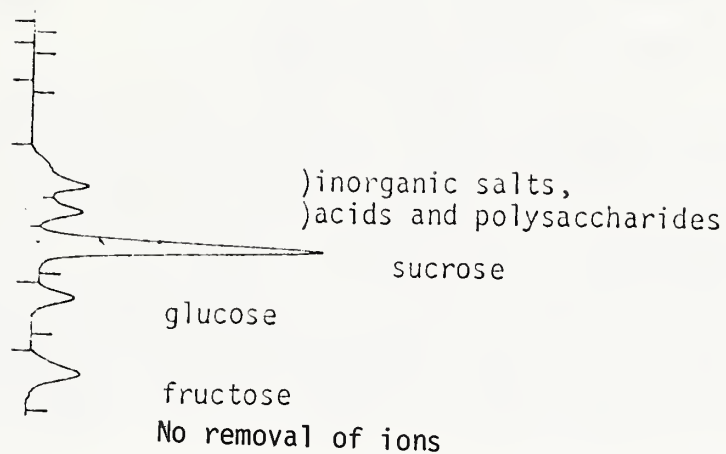


Figure 2.--GLC chromatogram of a final molasses sample.

Recently a precolumn de-ionising system has been described (Fitt 1984) that employs a short precolumn packed with a weak anion exchange resin in the hydroxide form and a strong cation exchange resin in the hydrogen form. This precolumn removed inorganic salts very efficiently and the chromatograms in Figure 3 show this by a dramatic visual difference. No sucrose hydrolysis was observed when these ion exchange resins were used.

3. Insoluble solids in samples (Alexander 1984)

Sample preparation in HPLC requires that each sample is filtered through a membrane filter (typically 0.45 μ). This filtration step can have serious implications when one compares results to GLC since the latter technique does not require any filtration steps. The net result is that in an HPLC analysis, the sample that has been filtered does not represent the original sample because it has been concentrated by an amount equal to the quantity of insoluble solids removed. Although the insoluble solids in factory samples



Removal of ions with a mixed bed precolumn

Figure 3.--Chromatograms showing removal of ions.

such as clear juice and syrup are not high, products such as cane final molasses can contain about 2% insoluble solids. This effect can lead to serious quantitation errors. An easy way to avoid the concentrating effect during filtration is to include an internal standard in the sample preparation prior to filtration. Then, as sucrose and the internal standard will both be concentrated by the same amount, the effect of the concentration during filtration will be effectively neutralised.

4. The influence of color in the sample

In a recent publication (Drushel 1983) it was shown that quantitation errors can occur if the sample to be analysed has a dark color and detection is by means of a differential refractive index detector. We have made a preliminary investigation into this effect (Hugo 1984) and have found that the color bodies present in factory samples elute in the general region where sucrose elutes. Although the color, when diluted, of samples such as cane mixed juice is light, the color of cane final molasses when diluted is still relatively dark, and thus must contribute to quantitation errors in HPLC. By using an appropriate resin, complete color removal can be achieved.

5. Sample concentrations

The concentration of sugars injected into the HPLC has been shown (Purchase and Walford 1984) to influence the quantitation of sucrose in mixed juice samples. Provided that the concentration of sucrose does not exceed 1.5% with a 10 ul injection, good quantitative data can be achieved. This effect is hard to explain but is presumably due, at least in part, to a combination of factors such as increased color, insoluble solids and inorganic salts discussed above.

EXPERIMENTAL (See Appendix for details)

Precolumn: The precolumn was made using a length of stainless steel tubing (150 mm x 2.1 mm ID), 2 u frits and appropriate end fittings. The internal diameter of the precolumn was kept as small as possible to avoid diffusion effects and thereby minimize band broadening and loss of resolution. The choice of resins to remove inorganic salts and acids has already been described (Fitt 1978). In order to remove the color from dark samples, a color removal resin (Amberlite IRA 958, Rohm and Haas) used in sugar refining was used. This resin is supplied in bead form but for our HPLC applications this bead form was ground into a finely divided form. In the latter form problems were encountered because the pressure needed for solvent flow was excessively high. In order to overcome this resistance to flow, the ground resin was mixed with silica (10 u) in the ratio 1 : 10. This modification reduced the precolumn backpressure to 40 atm while still being very effective in removing

colored components in samples.

Mobile phase: The mobile phase used was distilled, filtered (0.45 μ), and degassed water.

Instruments and column:

Chromatograph	:	Varian 5010 isocratic HPLC fitted with a column heater
Integrator	:	Hewlett Packard 3390A
Detector	:	Varian RI-3 differential refractive index detector
Column	:	Waters Sugarpak I (calcium form)

Instrument calibration: Instruments were calibrated by internal standarization (xylitol for the Waters Sugarpak I column). Three calibration standards were prepared containing sucrose in the range 0.7 to 0.9% sucrose (m/m). These three standards were injected before and after the samples and an average response factor calculated.

Sample preparation: Samples were prepared by weighing an appropriate mass of sample and internal standard and diluting with distilled water so that the sucrose concentration of the resultant mixture was in the range 0.9 to 0.9% sucrose (m/m). Prior to analysis each sample and standard was filtered through a 0.45 μ membrane filter.

Operating conditions:

Column	:	Waters Sugarpak I
Column temperature	:	85° C
Flow rate	:	0.5 ml/min
Column back pressure	:	100 atm with the precolumn

Detector stabilization: As the differential refractive index detector is very sensitive to small temperature changes, very good temperature control of the detector is needed to obtain accurate quantitative data. As sugar analyses using cation exchange columns require the column to be heated (85° C for the Waters Sugarpak I), the eluant entering the detector cell is at a higher temperature than the rest of the cell. This causes a slow baseline drift and can change response factors during the analysis of several samples (Mori 1985). The Varian RI-3 is fitted with a water jacket so that the cell temperature may be maintained constant using circulating water from a constant temperature bath. In our experience, a constant temperature water bath does not provide precise temperature control as it is successively heated and cooled leading

to baseline instability. In order to avoid this effect, a supply of compressed air was used as the coolant in the water jacket. Further, the transfer line from the column exit to the detector was cooled by passing the compressed air emerging from the water jacket along the length of the transfer line, by means of a piece of flexible plastic tubing. The flow rate of the compressed air was regulated until no baseline drifts were observed.

RESULTS AND DISCUSSION

All samples analysed in this study were final molasses samples. Since final molasses contains a higher proportion of impurities relative to sucrose than any other factory sample, any differences between results by different analytical techniques will be more pronounced. The samples analysed by HPLC in this study had been previously analysed by GLC as part of the Sugar Milling Research Institute's (SMRI) analysis of final molasses monthly composite samples for all South African factories. The comparative results by HPLC and GLC are listed in Table 2.

The results in this table show very good agreement with no bias. The data in Table 2 is presented graphically in Figure 4.

The regression equation for this data is

$$\% \text{ sucrose by HPLC} = 0.99996 \cdot \% \text{ sucrose by GLC} - 0.0055$$

with a correlation coefficient of 0.99995.

Table 2.--Comparison of HPLC and GLC for the analysis of sucrose in cane and beet final molasses

Sample	% Sucrose (m/m)		
	by HPLC*	by GLC**	HPLC/GLC
1	32.84	32.84	1.000
2	29.00	28.99	1.000
3	27.38	27.33	1.002
4	32.19	32.32	0.996
5	27.42	27.46	0.999
6	27.41	27.41	1.000
7	27.79	27.81	0.999
8	27.16	27.12	1.001
9	27.02	27.00	1.001
10***	30.46	30.51	0.998
11***	40.21	40.22	1.000
12	40.62	40.57	1.001
Mean	30.79	30.80	1.000

* Average of duplicates

** Average of triplicates

*** Beet molasses samples

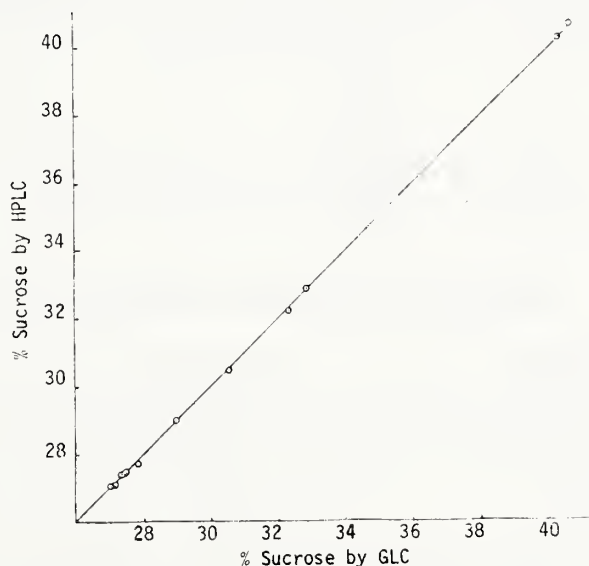


Figure 4.--Graphical presentation of data from Table 2.

Three samples analyzed in Table 2 (samples 7, 8 and 10) were used to establish the contribution of insoluble solids, ions and color to the overestimation of sucrose by HPLC. The contribution due to insoluble solids was determined by removing the color and ions using a precolumn and analyzing the samples by external standardization. We found it difficult to determine the influences of color and ions separately because the resins used to remove color also removed some ions and vice versa. Therefore, we determined the influence of ions and colors collectively by replacing the precolumn with a silica guard column and analyzing the samples by internal standardization. Tables 3 and 4 list the influences of insoluble solids, and of color and ions to the overestimation of sucrose.

Table 3.--Influence of insoluble solids and of color and ions to the overestimation of sucrose by HPLC

<u>% Sucrose by HPLC</u>			
Sample	Original analysis (Table 2)	Influence of insoluble solids	Influence of Color and Ions
7	27.79	28.35	28.58
8	27.16	27.80	27.64
10	30.46	31.64	31.10

Table 4.--Contribution of insoluble solids, and of color and ions to the overestimation of sucrose by HPLC

<u>Contribution to overestimation (%) *</u>			
	Insoluble solids	Color and ions	Total
7	0.56	0.79	1.35
8	0.64	0.48	1.12
10	1.18	0.64	1.82

* Absolute values

In order to determine the repeatability of the HPLC method to determine sucrose, three molasses samples (from Table 2) were analyzed on three different days. Table 5 shows the repeatability

is acceptable with an average relative standard deviation of 0.26%.

Table 5.--Repeatability of sucrose analysis by HPLC

Sample	% Sucrose by HPLC			Standard deviation	Relative standard deviation (%)
	Day 1	Day 2	Day 3		
1	32.84	32.88	32.75	+ 0.067	0.20
2	29.00	28.90	29.14	+ 0.121	0.42
3	27.38	27.30	27.30	+ 0.046	0.17

An obvious extension to this developed HPLC method is to quantitate glucose and fructose because knowledge of the concentration of these sugars provides valuable information for process control.

We have made a preliminary investigation into the quantitation of glucose and fructose in final molasses by HPLC (in the same analysis run as sucrose) and compared the results with the GLC method. These results are listed in Table 6.

Table 6.--Comparison of GLC with HPLC for the analysis of glucose and fructose in final molasses

Sample	% Glucose by		% Fructose by	
	GLC	HPLC	GLC	HPLC
1	7.7	7.6	9.0	8.8
2	7.9	7.6	8.9	8.7
3	7.5	7.3	8.1	7.8

This table shows that there is a definite bias, with the HPLC results for both sugars about 5% (relative) less than the GLC results. It is difficult to prove which method gives the most accurate result; but in our opinion, the HPLC method underestimates glucose and fructose.

The Hewlett Packard 3390A integrator was used for the integration of all peaks in the HPLC method. The area/height ratios (expressed in minutes) approximate the width at half height of the integrated peak. For good quantitative data the width at half height of sucrose in the standards and samples must be the same, and likewise for glucose, fructose and the internal standard. Table 7 lists the peak width at half height of sucrose, glucose, fructose and xylitol

for the three samples analyzed in Tables 5 and 6, and also the average for 3 standards.

Table 7.--Comparison of peak widths at half height

Sample	Peak width at half height (minutes)			
	Sucrose	Glucose	Fructose	Xylitol
Standards	0.425	0.620	0.653	0.722
1	0.424	0.596	0.711	0.723
2	0.424	0.593	0.727	0.721
3	0.423	0.608	0.739	0.724

The peak width at half height for sucrose is the same for both standards and samples and likewise for xylitol. On the other hand the peak widths for glucose and fructose show wide variations. This could be a cause for the underestimation of these sugars by HPLC as the elution characteristics of fructose and glucose in standard solutions are different from those in samples.

CONCLUSIONS

1. Provided adequate precautions are taken in the HPLC method, HPLC and GLC give equivalent analyses for sucrose in final molasses.
2. Analysis of monosaccharides showed that HPLC underestimates the concentration when compared to GLC.
3. Since final molasses is the most impure of factory samples, in terms of impurities relative to sucrose, analysis of factory products of higher purity should product similar comparative results.
4. The value of the precolumn used in this study is two-fold:
 - a) By removal of ions and color the accuracy is improved;
 - b) Because these interferences are removed, longer column life can be expected with fewer regenerations necessary.
5. Of great importance in HPLC is the temperature stability of the refractive index detector. The detector used in this study was hard to stabilize and surveys of detectors on the market should yield a better detector in terms of temperature stability.

6. More research work is needed on the comparison of GLC and HPLC for the analysis of monosaccharides.
7. HPLC provides a rapid tool for process control which will make interpretation of problems in process much easier.

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APPENDIX

Description of the HPLC method

1. Equipment and apparatus

Chromatograph	: Varian 5010 isocratic HPLC
Detector	: Varian RI-3 differential refractometer
Column	: Waters Sugarpak I (calcium form)
Mobile phase	: Filtered (0.45 u), distilled, degassed water
Flow rate	: 0.5 ml/min
Column temperature	: 85° C
Integrator	: Hewlett-Packard 3390A used in the peak height mode
Precolumn	: 150 mm x 2.1 mm ID stainless steel tubing, 2 u frits and end fittings
Precolumn packing material:	Equal masses of Bio-Rad AG3, 200-400 mesh (or equivalent); Bio-Rad Q-1505, 21-35 um (or equivalent) and Amberlite IRA 958 ground and mixed in ratio of 1 : 10 with 10 u silica.

2. Calibration standards

All sugars used must be pure and previously dried (in vacu) over phosphorus pentoxide.

Mass the following sugars (to the nearest 0.1 mg) into a 50 ml wide-mouthed conical flask:

	Sucrose (g)	Glucose (g)	Fructose (g)	Xylitol (g)	Water* (g)
S1	0.25	0.07	0.08	0.30	36
S2	0.30	0.08	0.09	0.30	36
S3	0.34	0.09	0.10	0.30	36

* The mass of water need not be recorded.

3. Synthetic molasses samples

Mass the following sugars (to the nearest 0.1 mg) into a 20 ml wide-mouthed conical flask:

	Sucrose (g)	Glucose (g)	Fructose (g)	Water* (g)
Syn 1	2.8	0.7	0.8	5.7
Syn 2	3.2	0.8	0.9	5.1

* The mass of water must be recorded to the nearest 0.1 mg.

Treat these synthetic samples like molasse samples as in (4) below.

4. Molasses samples

Mass the following components (to the nearest 0.1 mg) into a 50 ml wide-mouthed conical flask:

Sample mass (g)	Xylitol (g)	Water* (g)
1.0	0.30	36

* The mass of water need not be recorded.

5. Injection procedure

Inject the three calibration standards at the beginning of the analysis, then the molasses and synthetic molasses samples in duplicate, and finally the three calibration standards at the end.

6. Calculation

For each sugar (sucrose, glucose and fructose) calculate an average response factor using the six injections of the calibration standards. The calculation of the response factor for sucrose is shown in the example below:

$$\text{Sucrose response factor} = \frac{\text{height sucrose}}{\text{height xylitol}} \cdot \frac{\text{mass xylitol}}{\text{mass sucrose}}$$

Record the average response factor to three decimal places, and use it to calculate the percentage of sucrose in a sample as shown below:

$$\% \text{ sucrose in sample} = \frac{\text{height sucrose}}{\text{height xylitol}} \cdot \frac{100}{\text{sample mass}}$$

$$\frac{1}{\text{average sucrose response factor}}$$

A similar calculation procedure is used for glucose and fructose.

The synthetic molasses samples are used as a check on the internal accuracy of the HPLC method. In the calculation procedure a percentage for each sugar will be calculated which is then compared to the actual (massed) concentration. If these two percentages are not equal, then this provides a rapid routine indicator of systematic bias or instrument malfunction.

DISCUSSION

Mark Wnukowski, Amstar: I was curious whether or not you've examined the possibility of any inversion happening after the precolumn going into your separating columns on the HPLC.

Chorn: This precolumn has been published before. It's basically removing the ions from the product. In this original paper, the author goes into this aspect quite deeply and shows that there is no hydrolysis of sucrose. We did some of the studies just to verify that there is no hydrolysis of sucrose with the precolumn.

Wnukowski: How did you determine that--with GLC?

Chorn: No. You can take a standard sucrose solution and inject it into the precolumn and column and set the sensitivity of the detector in the monosaccharide region so that it will show up any hydrolysis products.

Wnukowski: I see that you were taking the GLC as being standard or as being the correct answer. I didn't see any recovery studies done on both instruments on the same sample. Did you run any tests like that?

Chorn: Well, if you go into the literature you will find that GC is an extremely well documented and validated technique. We took the GC results as proof and compared our results to that.

Stanley E. Bischel, American Crystal: Along the same line: I understand how you use the internal standard as far as particulates in filtration and I understand equal losses there, but did you use an internal standard when you deashed with the cation and anion resins? Did you test for the possibility, particularly with the monosaccharides, that fructose and possibly glucose had a greater degree of preferential sorption on these resins than possibly the standard did, or vice versa?

Chorn: If I understand your question correctly, I don't see how that can make any difference because you're dealing with the same chemical compounds that have the same behavior on the resins.

Bischel: But your internal standard is an example--it's not glucose or fructose.

Chorn: Right.

Bischel: Might it not be, and I believe there is some literature along this line, that on the resin, especially with carbohydrates, which are weak acids, you can have very small amounts of sorption, which without a lot of washing, can be irreversible; an anion resin, as an example. Is it possible that you could have had a

greater degree of absorption with your internal standard than possibly with glucose and fructose or vice versa?

Clarke: I have a point that might shed some light for the answers to Stan's question. We also sometimes treat the sample over these ion-exchange columns, but we do not collect the first bed volume off for analysis. The sample is allowed to run over the resin for 2 or 3 bed volumes and then the third or fourth bed volume is collected. Any absorption that's going to occur will have occurred in the first one or two bed volumes off, and so absorption will not affect the sample that is collected and analyzed.

Bichsel: That does answer my question.

John C. Williams, Tate & Lyle: Your peak width variations in the monosaccharides intrigue me. I have read papers on the history of HPLC that as the sugars and monosaccharides go down the HPLC columns, the different equilibrium species in solution can change and give peak broadening. Is this a thing you have considered? There are additives you can put in your solvent to prevent that.

Chorn: The column that we used was operated at 85° C and the reason for this is to make the differences in retention time between the alpha and beta anomers of the monosaccharides sufficiently close to produce one broad peak. The reason for the difference in peak width position is because in final molasses you have quite high ionic content and this will push the reaction in one direction or the other.

SYMPOSIUM ON HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
IN THE SUGAR INDUSTRY: A DIFFERENT ASPECT OF HIGH
PERFORMANCE LIQUID CHROMATOGRAPHY

Andrew M. Ho

Redpath Sugars, Ltd.

High performance liquid chromatography (HPLC) is an analytical technique that is well established in many industrial laboratories (Samuelson 1978, Brobst and Scobell 1981). It is also being used for process control and quality control by many sugar using manufacturers. Advances in column technology, high pressure pumping systems and detectors have transformed liquid column chromatography into a high speed, highly efficient method of separation. In recent years, it has started to become the method of choice for sugar analysis.

The liquid chromatographic system consists basically of a solvent delivery system, analyte injection apparatus, the chromatographic column, detector and recorder (Figure 1). The chromatographic column is a stainless steel column containing very small particles of narrow size distribution. The sample dissolved in an appropriate solvent, is applied to the head of the column in an even band with the help of the injector, and pure solvent is then allowed to percolate through the column via the help of a high pressure pumping system. Under the influence of the moving phase, the initial bands start to migrate through the column, and its components start to separate. Those components only weakly held by the absorbent pass rapidly through the bed, those more strongly held, more slowly, etc., until a complete separation of all the constituents of the mixture is achieved. When the components elute from the column, various detection systems may be used to detect and record the results. Detectors such as refractometers, ultra-violet spectrophotometers, fluorimeters and mass detectors (Verbaar and Dirkx 1977, Kunberger et al. 1983, Macrae and Dick 1981, Honda et al. 1980) have been used and various degrees of success have been achieved. Considerable effort has also been made in improving the detection sensitivity and efficiency of the analysis by the use of post-column derivatization (Thompson 1978, Russell et al. 1979) and solvent modification (Verbaar and Kuster 1981).

HPLC has come a long way and the literature is flooded with papers, reporting advances in separations and techniques. In fact, more and more methods are now becoming "Standard Methods."

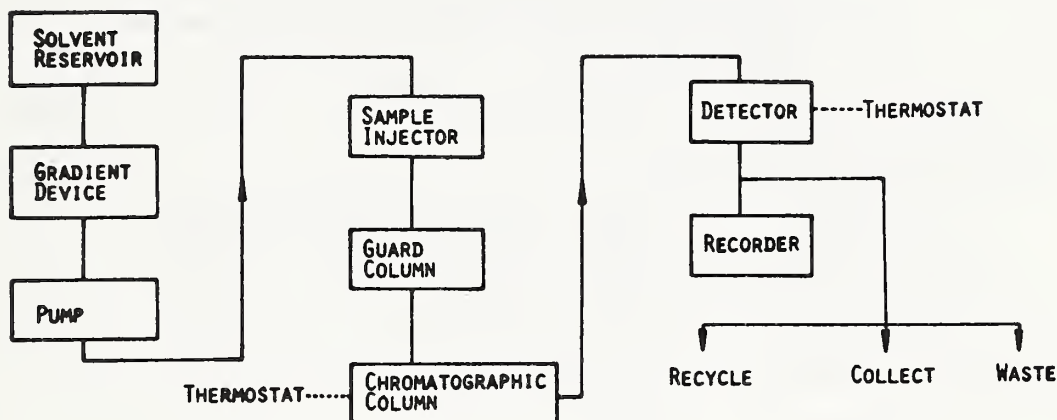


Figure 1.--Functional schematic of a modern high performance liquid chromatographic system.

I am not implying that HPLC will solve our problems; I am not suggesting that everybody go out now to start buying HPLC equipment, or asking the engineers to start building large chromatographic columns in the refineries. I only wonder sometimes why so few sugar factories and refineries today are using HPLC as part of their daily analysis, while many of our competitors (corn people) and customers now possess the equipment in their laboratories and are using it to examine our products. Maybe it is because we feel that HPLC does not offer the precision we want; maybe it is because we do not believe that it has the accuracy of the more traditional classical methods; maybe the instruments are too expensive, or simply because we are being too cautious to do anything.

There are some studies done on comparing sugar analysis results obtained by HPLC analysis and that obtained by classical titration methods (Brown and Sharp 1984, Clarke and Brannan 1979). Unfortunately, no conclusive results have been obtained. In our laboratory, we have tried some similar experiments and I would like you to comment on them.

The HPLC system we used is as follows:

SYSTEM: Waters Associates HPLC system Model 6000A
Valco Fixed Volumn Automatic Injector
Waters Associates Differential Refractometer
Model R401
Waters Associates Data Module

HPLC Operating Conditions:

Column: BioRad Aminex HPX-87C Carbohydrate
300 x 7.8 mm @ 85° C

Solvent: DDI water @ 60° C

Flowrate: 0.6 ml/min

RI Range: 16x

Chart Speed: 0.25 cm/min

Injection Vol: 10 ul

Guard Column: a) Carbohydrates @ 25° C
b) Cation & Anion @ 25° C

We start off by examining the precision of our instruments. A sample containing approximately 50% sucrose and 50% invert sugars of high purity was used. Ten injections were done in succession and the peak areas were evaluated by the Waters Data Module. The results obtained were quite good with a coefficient of variation (C.V.) of 0.30% for sucrose to 0.56% for glucose (Table 1).

After the precision had been established, we proceeded to analyze some "Synthetic Sugar Syrups." We prepared the syrups by weighing out exact amounts of each sugar (Table 2) and dissolved them in deionized distilled water. Duplicate results were obtained (Table 3).

Table 1.--Precision studies of HPLC carbohydrate analysis

	Standard Deviation	Coefficient of Variation
Sucrose	0.15	0.30
Glucose	0.14	0.56
Fructose	0.09	0.36

Table 2.--Concentration (gm/10 ml) of sucrose, glucose and fructose in "Synthetic Sugar Syrups"

Sample Number	Sucrose	Glucose	Fructose	%Suc	%Glu	%Fru	%Invert
1	3.8040	1.9000	1.9000	50.02	24.99	24.99	49.98
2	3.4232	2.0900	2.0900	45.02	27.49	27.49	54.98
3	3.6050	1.8025	1.8025	50.00	25.00	25.00	50.00
4	3.8027	3.8012	0.0000	50.01	49.99	0.00	49.99

In the past, we have always concentrated on the precision of the HPLC method. We used the classical Lane and Eynon titration method as a reference and we compared its precision with that of the HPLC. We used the titration method as a standard even though we know it will indicate higher values if there are non-sugars which react with the alkaline Cu(II)-complexes in the sample, we know that the method should never be used for the determination of reducing sugars content of beet molasses because of interferences, we know that we should use different tables for glucose and fructose, and in fact even when different concentrations of sucrose are present. Without knowing the exact proportions of the various sugars in a sample, the titration method may be very precise, but not necessarily very accurate. Now let us examine Table 5, which is a comparison of five actual G-50 Invert Syrups. Analysis shows a difference of about 3.3% in the amount of invert sugar found, using the two methods, ranging from 2.57% to 4.95% on solids. In our case, analysis by titration appears to give a lower invert concentration (or is it that analysis by HPLC gives a higher invert content?). A further examination of the analysis of the "Synthetic Sugar Syrups" (Table 4) using the two methods indicates the HPLC had a 99.6% to 101.0% recovery while the classical titration method reported a 91.4% to 94.8% recovery. If we now take the average of the percent recovery by the titration method and use it as a correction factor, and apply it to the analysis of the five actual invert syrup samples, we find the difference between using the two

methods now ranging from only -1.06% to +0.33% with an average of -0.40% (Table 6). It is unfortunate that we did only a few samples, so, statistically, such figures cannot be universally accepted. But I do believe that with more collaborative studies, a much better correlation can be found.

Table 3.—Recoveries of sugars from "Synthetic Sugar Syrup"

Sucrose	Theoretical	HPLC	%Recovery	Mean	S.D.	C.V.
Sample 1	50.02	49.43	99.00			
Sample 2	45.02	44.79	99.49			
Sample 3	50.00	51.19	102.38	*100.29	1.83	1.82
Sample 4	50.01	50.25	100.48	**100.34	1.49	1.49
Glucose						
Sample 1	24.99	26.48	105.96			
Sample 2	27.49	28.94	105.27			
Sample 3	25.00	26.39	105.56	*105.60	0.35	0.33
Sample 4	49.99	49.75	99.52			
Fructose						
Sample 1	24.99	24.00	96.04			
Sample 2	27.49	26.47	96.29			
Sample 3	25.00	23.41	93.62	* 95.32	1.46	1.53
Sample 4	0.00	0.00	—			
Invert (Glucose + Fructose)						
Sample 1	49.98	50.48	101.00			
Sample 2	54.98	55.41	100.78			
Sample 3	50.00	49.80	99.60	*100.46	0.75	0.75
Sample 4	49.99	49.75	99.52			

* Calculated from Samples 1 to 3

** Calculated from Samples 1 to 4

Table 4.--Analysis of "Synthetic Sugar Syrups" by HPLC & classical method

Sample Number	Theoretical % Invert	Sucrose	Glucose	Analysis by HPLC			Analysis by Titration	
				Fructose	% Invert	% Recovery	% Invert	% Recovery
1	49.97	49.52	26.48	24.00	50.48	101.0	45.67	91.4
2	54.98	44.79	28.94	26.47	55.41	100.8	52.14	94.8
3	50.00	51.19	26.39	23.41	49.80	99.6	47.33	94.7
4	49.99	50.25	49.75	0.00	49.75	99.5	47.42	94.8

Table 5.--Analysis of actual invert syrup samples (G50) by HPLC & classical method

Sample Number	Sucrose	Glucose	Fructose	% Invert	Analysis by Titration	Diff.
					% Invert	
5	42.85	29.47	26.50	55.97	53.4	+2.57
6	44.85	28.43	25.59	54.02	50.8	+3.22
7	44.83	28.31	25.41	53.72	50.7	+3.02
8	45.88	27.99	25.05	53.04	50.2	+2.84
9	26.34	40.08	32.77	72.85	67.9	+4.95

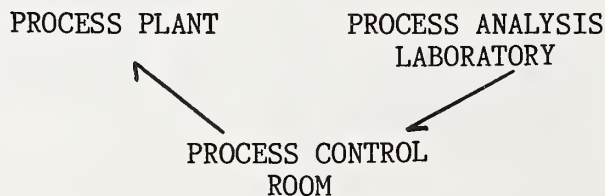
Table 6.--Analysis of actual samples by HPLC and titration with use of a correction factor

Sample Number	% Invert by HPLC	% Invert by titration w/o correction	% Invert by titration with correction
5	55.97	53.4	57.03
6	54.02	50.8	54.26
7	53.72	50.7	54.15
8	53.04	50.2	53.62
9	72.85	67.9	72.52

Another interesting observation was also revealed by this experiment. In the analysis of the "Synthetic Sugar Syrups," although the same amount of glucose and fructose were used, results show a higher concentration of glucose than fructose, with a G/F ratio of approximately 1.1. Interestingly, the percent recovery of invert (glucose + fructose) remains at approximately 100%. If we look at Synthetic Syrup Sample 4, where no fructose is present, recovery of glucose is about 100%. Looking at the five actual samples, a similar situation exists. All five samples show a higher glucose concentration than fructose and the G/F Ratio are all around 1.1. We do not know exactly how or why this happened.

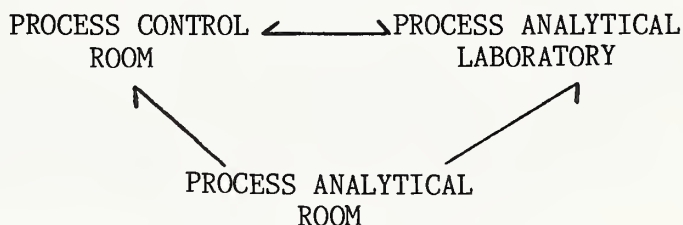
There was no apparent inversion of sucrose under the operating conditions. We also analyzed samples with different ash contents and similar results were obtained. I hope future studies will help us solve this mystery.

Besides being used as a quality control device, HPLC may also be used as a process control device. In our refinery, we manufacture invert sugar syrup by inverting liquid sucrose with HCl and when the estimated invert reaches the desired percentage, the syrup is neutralized and pumped to the storage tanks. To find out exactly how much inversion has taken place, samples must be obtained from the process plant and brought to the laboratory for analysis which takes at least 20 minutes. The data obtained from the analysis are then communicated to the process control room where adjustments to the process will be made based on the data obtained:



Since the residence time of the syrup in the reactor is only about 20-30 minutes, such lengthy procedures will allow adjustment only to the third batch. As a result, shut down and loss of production do occur at times. Due to the amount of samples that have to be analyzed by the analytical laboratory, the invert syrup produced by the reactors can only be determined one or two times an hour, thus making the process even less efficient.

At the moment, we are contemplating using HPLC as an in-line process control. The theory is if the invert in the reactors could be determined on a continuous basis, a more efficient operation may be devised. The frequency of analysis can be increased by automatically sending a sample of the reaction to the analytical instrument which is dedicated to the analysis of invert in the process stream. Data obtained from the analysis are automatically transferred to the process control room so that the adjustments can be made. The analytical laboratory also receives the data for verification and record keeping:



In the most ideal situation, adjustments will be made by passing the analytical data directly to the process control computer.

To use the HPLC system as an in-line process control, first of all, we will have to find a column that will provide very rapid analysis. A new column on the market known as the "Fast Carbohydrates Analysis Column" will provide an analysis in only about 4 minutes (Figure 2).

The next step is the question of transferring the sample. During the invert syrup manufacturing process, the samples of the process stream are collected by the operator and hand carried to the laboratory for analysis. With in-line analysis of the process stream, samples are transferred to the HPLC system via specialized plumbing and pneumatically activated valves. Figure 3 shows the plumbing configuration and the sample valve sequences needed for a single in-line carbohydrate analysis system.

Instrument Calibration

1. Activate Valve No. 4 for Calibration Standard Solution.

Calibration Standard flow through valve No. 4

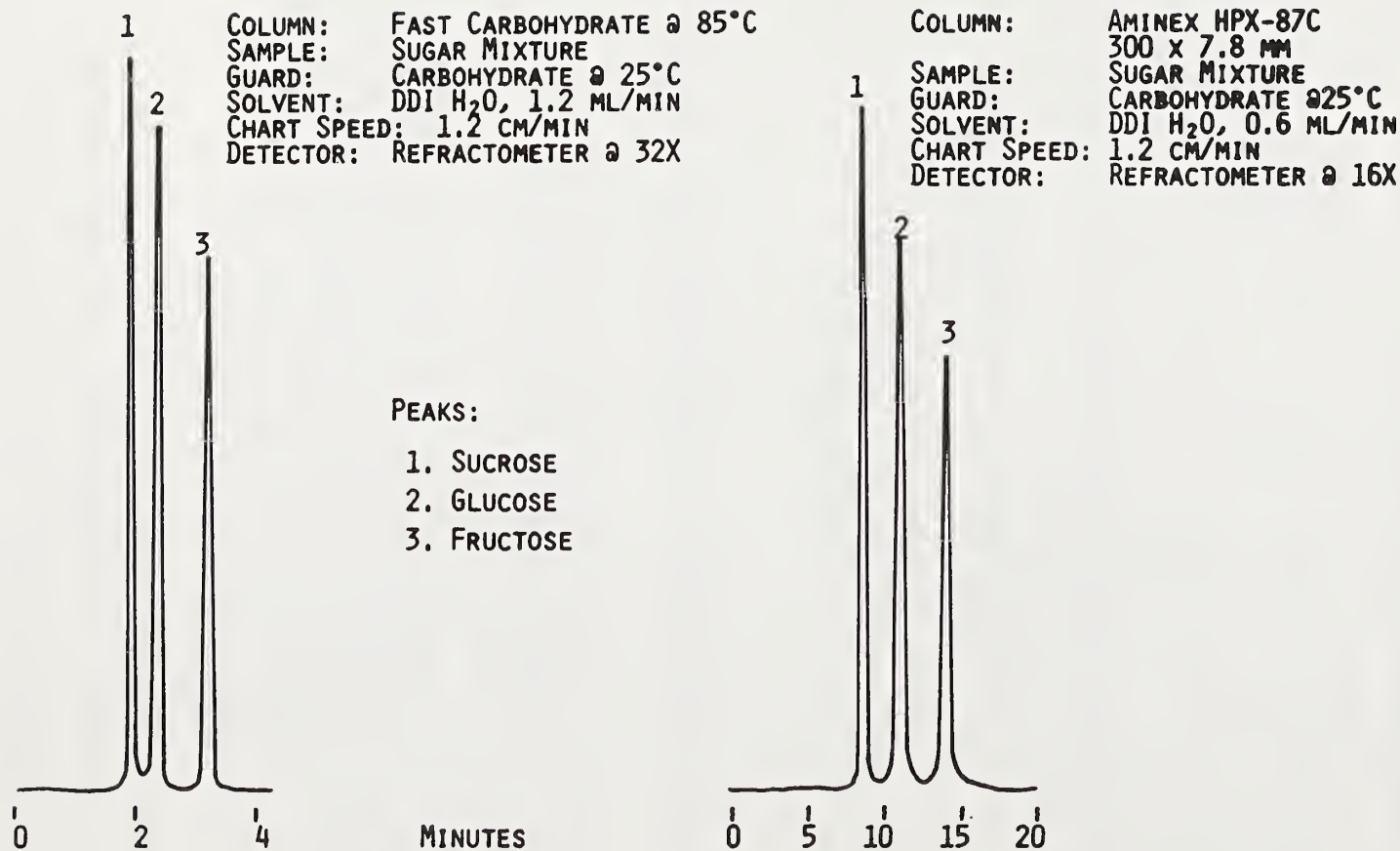


Figure 2.—Comparison of analysis times and separation of sugars on fast carbohydrate column and Aminex HPX-87C columns.

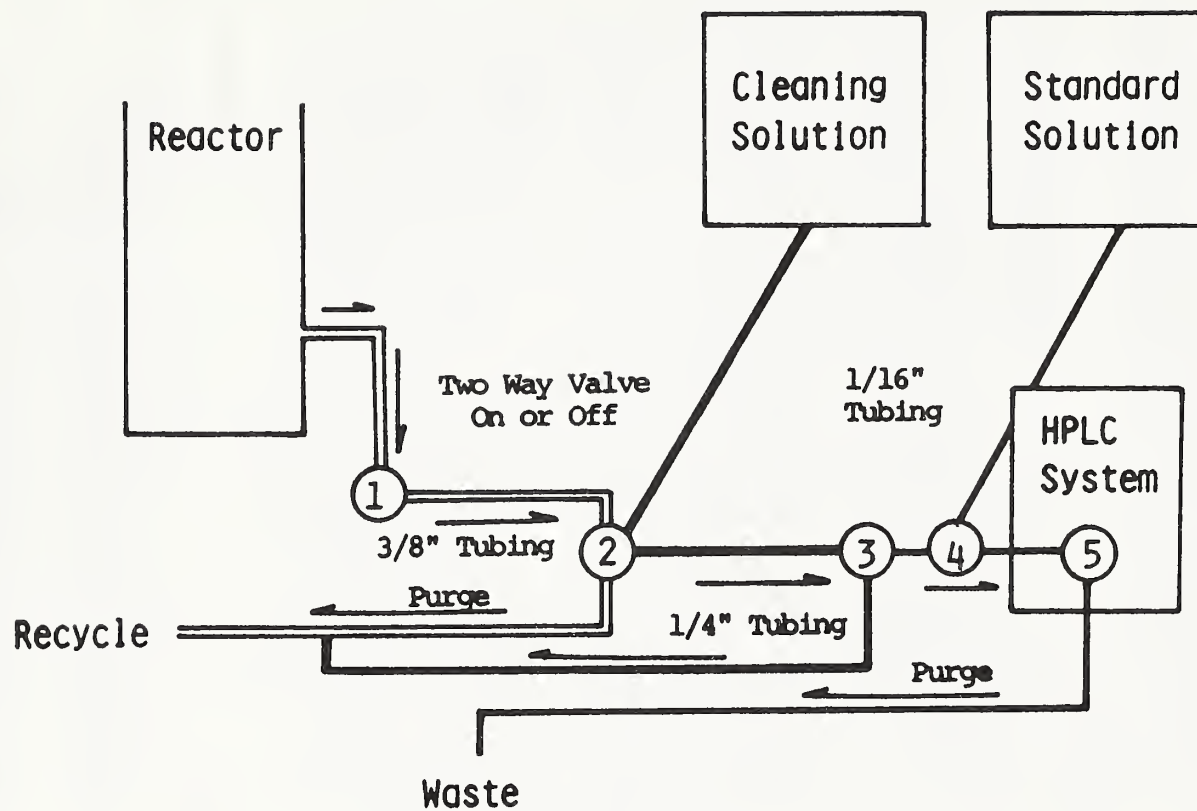


Figure 3.--Plumbing configuration and sample value sequences needed for a single in-line carbohydrate analysis system.

Calibration Standard will be held in a pressurized stainless steel tank to eliminate the need for a pump.

2. Activate Valve No. 5 (Sample Injector)

Based on the data obtained with this sample the instrument is calibrated.

A single calibration per each 8 hour period is all that is necessary.

Operator has the option of calibration at any time.

Process Analysis

3. Activate Valve No. 1 (Reactor to Sample Line)

Sample transfer involves two operations:

1. Purging the sample line with fresh sample.
2. Reducing the diameter of the plumbing from a 6" process line to 1/16" HPLC tubing.

Fluid from the process line is purged through the 3/8" sample line with enough sample to equal three times the volume of the sample line. With Valve No. 1 open, Valve No. 2 is activated to allow purging of the 1/4" sample line.

4. Activate Valve No. 2 (3/8" Sample Line to 1/4" Sample Line)

With Valve No. 1 open, Valve No. 2 is switched from purge through the 1/4" line position. Fresh sample is purged through the 1/4" sample line to equal three times the volume of the sample line.

This sample sequences is also necessary for the introduction of System Cleaning Solution. Excess sample goes to recycle.

5. Activate Valve No. 3 (1/4" Sample Line to 1/16" HPLC Tubing)

Valve No. 1 is open, Valve No. 2 is in the 1/4" position, Valve No. 3 is in the purge position and Valve No. 4 is open to the Sample Line.

Fresh sample (Process sample or calibration sample) is purged through the 1/16" HPLC tubing to equal three times the volume of the 1/16" tubing.

At this point in the sampling process, the sample is passing through the HPLC system injection valve.

6. Activate Valve No. 5 (HPLC Sample Injector)

Prior to the activation of this valve, all sample lines have been purged with fresh sample. For the calibration of the instrument only the 1/16" HPLC tubing has been purged (Valve No. 4). Calibration standard and all excess process sample go to waste.

Valve Sequence Control

Value operating sequence up to the HPLC system should be controlled by the process control operator. The Calibration Standard and the HPLC injection valve can be controlled by the HPLC system. Using a micro-computer (e.g. Apple IIe or IBM PC) and the automation interface, a signal (contact closure or High/Low Voltage) can be used to activate the HPLC injector or the Calibration sample valve. In the final configuration a special timer could be installed to control all the valves in the process analysis system. Based on the sample line purge time of 1 minutes and HPLC analysis time of 4 minutes with one HPLC system for every 3 reactors, each reactor could be analyzed every 15 minutes. During preliminary testing of the system the exact time needed to purge a sample line would have to be determined. At Redpath, we are contemplating using such a design for our invert syrup manufacturing process, and a small laboratory scale model will hopefully be built and tested in the near future.

Finally, I believe HPLC is a very useful technique, and can be used in the sugar industry in more than one way. We have encountered a lot of problems in analyzing many syrups and samples; but if we invest enough man-power, time and money, I believe the technique can give us more than we we bargained for.

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SYMPOSIUM ON HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN THE SUGAR INDUSTRY

Robert F. Hutton

Consultant

I want to begin by augmenting Andrew Ho's comments on the use of liquid chromatography as a process control method. It certainly is being used and is being advanced by many industries. An area of particular interest is the fermentation industry where fermentation traditionally was followed by the disappearance of a carbon or nitrogen source, or the appearance of a metabolite, without real knowledge of the intermediate processes, their inhibitions and reversals. The ability to do rapid liquid chromatography, in that instance, permits one to profile chemically the fermentation broth as it develops. A difficulty there--and it is a general problem with process LC--is getting a sample of clarified liquid, of pure supernatant. One of the techniques that holds promise is tangential flow filtration, where the filter does not readily clog, and a continuously clear supernatant can be obtained.

Figure 1 further supports what Andrew Ho has said. This figure was kindly given to me by Kenneth Cohen of IBM Instruments (L.C. division), who showed it in a presentation at an L.C. symposium that I chaired in Boston earlier this week. The diagram points out the state-of-the-art in analytical separations: it is a demonstration chromatogram of six various compounds. These six compounds, with the exception of one pair, are base line separated in one minute. This is done on a three micron particle column. The question was, "What next? Two micron particles or one micron particle? A faster analysis?" Dr. Cohen answered that in terms of ordinary laboratory analyses, the analytical speed here far exceeds the sample preparation speed, and that the rate limiting step in analysis is now sample preparation. There really is little need to get any faster, except in the area where further speed might be useful: the area of process control.

The next subject, my main topic, is the area of large scale chromatography. I feel a little presumptuous talking to this group about purification on a large scale since you purify enormous

tonnages to a high degree of purity, usually without the benefit of chromatography. Chromatography, however, is becoming an increasingly important technique to a number of industries, and although I cannot advise you to run into the factory and start installing large-scale chromatographs, I want to tell you something about what capabilities exist and where it's heading, so that you may develop an idea of the preparative or process scale chromatograph in your armamentarium of new opportunities. Only a few years ago, large-scale chromatography on a very rapid time scale, like that of analytical chromatography, would have given very poor results. But in the last couple of years, large-scale chromatography, particularly in the area of substances of a high value, has developed tremendously.

Preparative chromatography has different aims from analytical chromatography. In analytical chromatography, the objective is to find out how many things are present and how much of each, and you get the best opportunity for quantitation with the removal of interferences, that is to say, complete separation. So there is baseline separation and sharp peaks. Preparative chromatography is very different; the objective is to isolate something, and the critical consideration is throughput: getting the most through as cheaply and as quickly as possible. The appearance of the chromatograms are very different from those in analytical chromatography, and much less aesthetic. By the standards of analytical chromatography, there are not very many good-looking peaks. A typical analytical chromatogram of a pair of positional isomers, under typical conditions on a standard analytical column of 4 mm by 30 centimeters, with 100 microgram load of material, shows two peaks, nicely separated, as shown in Figure 2. When the load is scaled up to something reasonable that you can see on the same column, instead of 100 micrograms, that is the end of the aesthetics. With as little as 54 milligrams of sample, the column becomes dramatically overloaded as shown in Figure 3. In analytical chromatography, much underloaded columns are very effective and very efficient. In preparative chromatography, the best bet is actually to operate in a highly overloaded condition. One needs to go to a larger column, clearly, for more separation capability: from this typical analytical column, 30 centimeters long by 4 mm in diameter, we scale up to 57 mm in diameter, almost 15 times the diameter of the analytical column, and about the same length. With a column of that size, one can put on a sample of 10 grams, and get something that looks like a chromatogram, although there is not complete separation, as shown in Figure 4. The detector, in this case a refractive index detector, sums two curves, so that the valley between the two curves appears to be less good than it actually is. On the front part of the first curve, there's pure material and on the back part of the second curve there's pure material, and in between there's some degree of mixture. If you make chromatographic cuts at fraction 2 on Figure 4, you can get pure material, as with fraction 4. In between 2 and

4, you can also make cuts depending on what degree of purity or what degree of impurity you are willing to accept. We can then look at fractions 2 and 4 under analytical conditions and see how successful that separation is. The time scale, in going from typical analytical conditions of 100 micrograms to a preparative scale of 10 g is essentially the same. The analytical separation was on the order of two minutes; even going up to a large scale, separation is still somewhere in that range. In general, by going to larger columns and pump systems with high-flow rates, the time frame for separation in the analytical mode can be maintained with the preparative. The analytical chromatograms of the separated fractions show that even without the sharp baseline separation, one can operate in a rapid time scale and get good separation.

The next question is, what systems are available? There has been a certain amount of work going on in universities which is not generally known to the public, and one of the finest practitioners is Professor Michael Ladisch, who has been doing some excellent work in the area of large scale separations. There has been some commercial instrumentation appearing in the last few years. The systems essentially are all the same: a column which does the separation, a pump which pumps the mobile phase through it, and a detector of some sort. In general, at very high flow rates and very large volumes, the entire mobile stream is not sent through the detector, as in an analytical system. There is some sort of flow splitter, sending 1% or 0.1% of the stream through the detector, and the rest into a collection device.

In large-scale chromatography, there are various different approaches to making a chromatographic packed bed, that is, making columns. One of the earliest columns, developed by Waters Associates, is a plastic cartridge, put into a capped, steel compression chamber. In the annular space between the outer wall of the cartridge and the inner wall of the steel casing, pressure is applied, in this case pneumatically, at about 35 atmospheres.

The first systems of this type have been extended from what is essentially a bench top instrument, which uses flow rates of about maximum 500 ml/minute, into a large process scale instrument shown in Figure 5. These large columns are also radially compressed, either by pneumatic or hydraulic means, with a plastic cartridge inside those steel cylinders. Waters' approach is to use short columns (these are either 6 or 8 inches in diameter and 2 feet in length) in series so that the largest system at present is 6 or 8 inches in diameter by 6 feet in cumulative length. Their approach to using combined short lengths has some special operational features. This is now a commercial process chromatograph; there are several in place, used for very high-valued products, like pharmaceuticals. One is in place at Smith-Kline in Philadelphia, for instance.

Another approach to column technology is that of a French engineering company, Elf-Aquitaine, a subsidiary of which is involved in liquid chromatography, Elf Technologies. Elf, long accomplished in process scale gas chromatography, is now moving into process scale liquid chromatography. Their approach is to use what is called axial compression to form a chromatographic bed: a cylinder forms the outer wall of the chromatographic column, and contains a movable piston, which has in it a fritted disc so that liquid can flow through it. Packing material is in the column, perhaps as a slurry, and as the piston drives up, the packing is driven up and compressed while the liquid flows out through the fritted disc. Pressure is maintained on the packed bed from the bottom and so this is an axial compression rather than a radial compression. To unpack the column and change the packing material, the lid is removed and the piston run up, pushing everything out. To make such a column, a slurry of the packing material of choice is held in a reservoir, the piston is pulled down to suck this in, then the column is capped, the fritted disc attached, and the sequence described above of pushing the liquid out and compressing the bed is followed. Elf has several models: their largest is about 2 ft in diameter by about 14 ft in length, as shown in Figure 6.

Another example is a separation of a couple of rather expensive steroids: A 1 kilogram injection is done on a column of 40 kg of standard Merck silica, under normal phase conditions, in dichloromethane and THF, with UV detection, and at two flow rates: at 30 l/h and at 130 l/h. Essentially an analytical loading is on this huge column, very underloaded, with a nice, clear separation. At 1 kg at 130 l/h, one expects a deterioration of the picture, and one gets that. At 130 liters per hour, there is almost complete separation on a kilogram in an hour. This is a fairly difficult separation because these two compounds differ only by one double bond; otherwise, they are identical.

With regard to the cost of this kind of thing: if you want 100% purity, at 30 liters per hour, you can get 100% purity of each of the two components, and you get 99% of the leading one and 83% recovery of the trailing one. If you're willing to accept lower percentages, if you don't need 100% purity, if you'll take say 96% purity of each one of them, then you can get 100% recovery of each one. The loss of recovery occurs where the compounds are mixed and are incompletely separated. If you want to scale up to 130 liters per hour, then you must give up some purity or recovery for the added throughput. You can have 100% purity with 98% recovery of the first component, but to get 100% recovery of the trailing one, you will only get 21% recovery. Or, you can get 84% and 12% purities with 100% recovery of each component. Those are the trade-offs you make. That's not so much chromatography as dollars and cents.

A third approach to operational large scale chromatography is by a relatively young company called Separations Technology, based in Peacedale, Rhode Island. This company has very academic origins: it started as one person, a graduate student, who had a Waters Prep 500 instrument. He decided that the columns were too expensive, and that he would make one of his own knowing full well he would never get, he thought, the resolution of the commercial columns. He made one, and got about as good resolution as the commercial one; so, he then started making the columns commercially. His next step was to make a chromatograph to use the columns on. His columns have neither radial nor axial compression. They are standard rigid wall columns that can be packed in 2 ways. Dry packing can be shaken into a big column to try to get a homogeneous bed; liquid is then pumped through to settle the material. The head space that has been formed by settling is filled up with some more packing material. The other way is to do it the way that analytical columns are generally made, which is slurry packing under high pressure, driven into the column. Separations Technology columns go up to 8 in in diameter and 6 ft in length, hold about 35 kilograms of packing material and will take loads of about 1 kilogram of material.

A feature of all preparative instruments is the concept of recycle. The normal fate of some material being chromatographed is this: It's injected into the flow stream by an injector, carried along by solvent from the pump, and through the column. In analytical separations, if the chromatographic bands are not baseline separated, one starts over on a new separation. But, if there isn't baseline separation, what can be done aside from changing the mobile-phase makeup? The first thing is to make the column longer--a rather costly thing to do. Double the column length means double the cost, double the back pressure and double the elution time--not an entirely satisfactory way to go. Double the column length, does not actually double the resolution because the efficiency enters as a square-root term, so the doubling only increases resolution by 1.4. But here, recycling can improve the separation. A pair of chromatographic bands which exit the column can be carried back right to the head of the column, and put through the same column for a second time, through a pump. This can be done on a small to medium scale and is an established technique, and a feature of all the chromatographs mentioned in this paper. These bands can be recycled around the columns as many times as is necessary to get the desired separation. Figure 7 shows an example of a recycled separation done on the axially compressed Elf column. The mixture contains two optical isomers, two enantiomers. At one pass, there's hardly any separation at all; after four passes, there's partial separation; by 10 recycles, there is nearly complete separation. That's a lot simpler than buying 10 columns.

What might this technique be used for? This is especially useful for separation of high-value products. I was quite provoked by

Mary An Godshall's talk on various flavor components that are in brown sugar. There is a mixture there; and it could be possible to select out chromatographically individual flavor components in order to direct the flavor in one way or another.

I was approached recently to consult on a problem in a very different area of food product. In its processing, this product is clearly separated into two things--what is considered to be the edible part and what is considered an inedible part. The inedible part is not inedible because it's fibrous, it just has some tastes in it that are generally regarded as unpleasant. An analysis of this inedible part shows, of course, many components in it. There is good evidence that a number of these components are extremely desirable flavor and taste components but are overwhelmed by one or two overridingly unpleasant characteristics in the mixture. The proposal is to separate this inedible material, on a very large scale, to remove the several, very desirable flavor components for reblending with the edible part. This could tune the product for different markets. The separation of essential flavoring components or essential oils, which have distinctive flavor or taste qualities, with removal of unpleasant things, could be applied to custom blending of brown sugars or other food products. I remember visiting a cognac house in France, where there were many different spigots representing the blending components of the brandy. They blended their cognacs for different markets. One went to South Africa, a different blend for the U.S. market, a different for French consumption and so forth. Quite apart from alcohol content, the flavor makeup of all these cognacs is quite different. One might want to isolate high flavor, high-value components.

A very recent development is that β -cyclodextrin, which is 7 glucose residues, is actually being isolated and purified to be used as a chromatographic support. The β -cyclodextrin forms a helix and has a hole of a certain size in the center. It's very useful as a size separation medium in chromatography, attached to some support. If β -cyclodextrin molecules are hitched on to silica, chemically, they provide a chromatographic column which will act not only as a sizing column but, because that cavity is formed by a molecular spiral in only one direction, it also has chirality or handedness, and it's useful therefore in separation of optical enantiomers.

One other possibility has occurred to me, listening to a number of the papers in the last couple of days: several had to do with large molecules and furthermore, mixtures of large molecules which are sometimes not well-defined. Traditionally, with mixtures of large molecules, these are only two kinds of polymers: either synthetic polymers or polymers of biological origin. Until recently, chromatograms of synthetic polymers, or of polymers where all of the molecular species are not the same, showed one big peak,

which is in fact the envelope of a number of peaks which columns are incapable of resolving because the polymers are too similar for the columns to tell apart. The newly developed types of chromatographic columns are far more discriminating. Let me give you two examples. If you're looking at polymers, even very high molecular weight polymers, or large molecules of biological origin in which the molecules are all the same, as they are, say, in a protein, you can get the kinds of separations that you used to see only for small molecules. Figure 8 shows an example of protein separated by size on a gel permeation or size exclusion column. Peaks are relatively sharp because species are unique. What about polymer separations when all the chain lengths aren't exactly the same? There are encouraging developments in that case too. Figure 9 shows an absorption separation, not a size separation, of a polytetramethylene ether. The repeating group is four methylene groups and an oxygen. At either end of the chain is a hydroxyl group. The lower polymers separate well, and there is still distinct separation between the 40-mer and the 41-mer, and possibly even between the 45-mer and the 46-mer. This corresponds to separation of a molecular weight of 3520 (the 40-mer, with $N = 40$) and 3608 (the 41-mer), which is a high degree of discrimination.

In complex carbohydrate mixtures, where one might want, for instance, the α -, β - or γ -cyclodextrins, there may be unique properties to a particular polymer which might have, let's say, interesting biological activity. This would again be a high value product. There is now at least the possibility of separating such things chromatographically on a scale that might be economically feasible.

Column: octadecyl, IBM, 50x4.5 mm, 3 micron
 Mobile phase: 70/30 acetonitrile/0.1% H₃PO₄
 Flow rate: 1.6 ml/min, column temp. 24.9°C
 Detector: Variable UV, 254 nm, IBM 9523, 0.20 AUFS

- 1 uracil
- 2 acetophenone
- 3 methylbenzoate
- 4 diethylphthalate
- 5 toluene
- 6 ethylbenzene

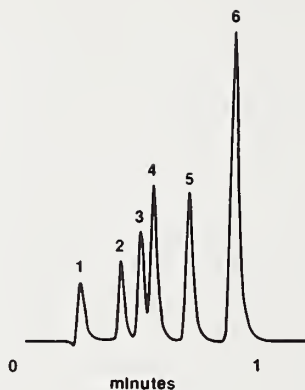


Figure 1.--Baseline separation of six compounds in one minute on a 3 micron particle column.

ANALYTICAL SEPARATION OF TETRAHYDROPHENANTHRENE MIXTURE

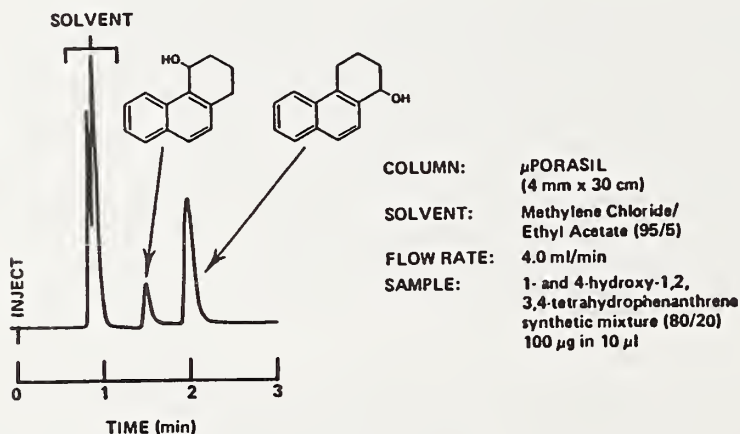


Figure 2.--Analytical separation of positional isomers.

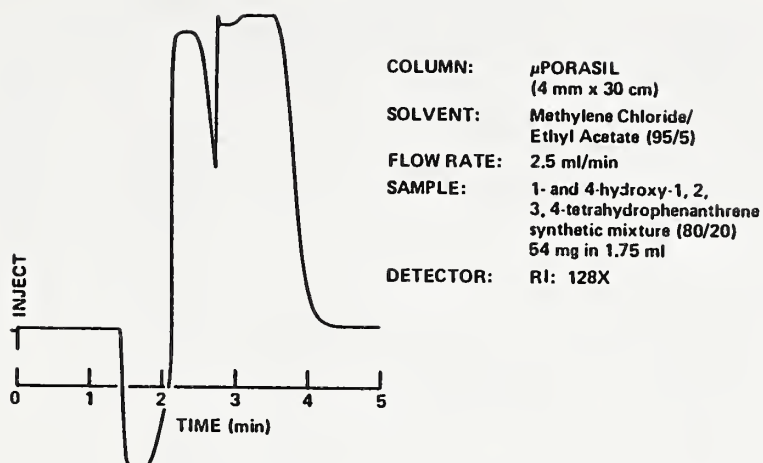


Figure 3.--Example of an overloaded column.

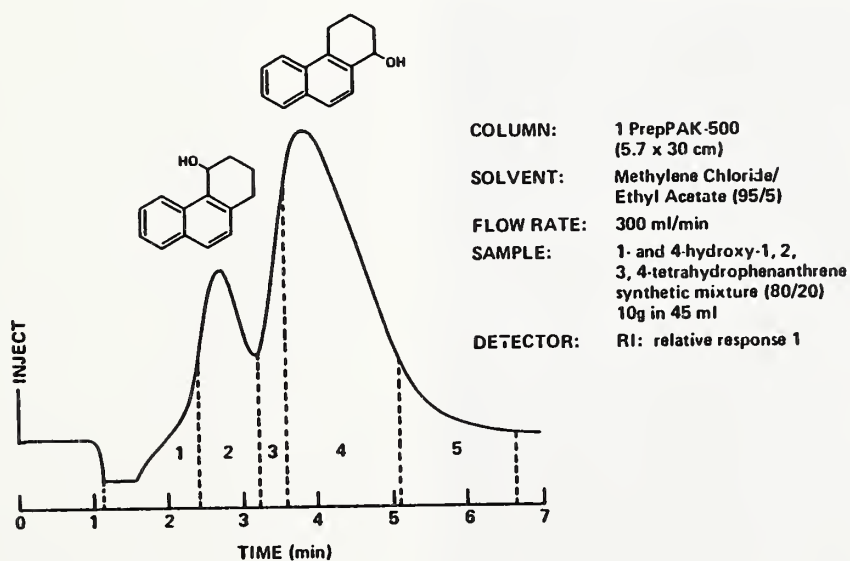


Figure 4.--Example of a preparative separation.

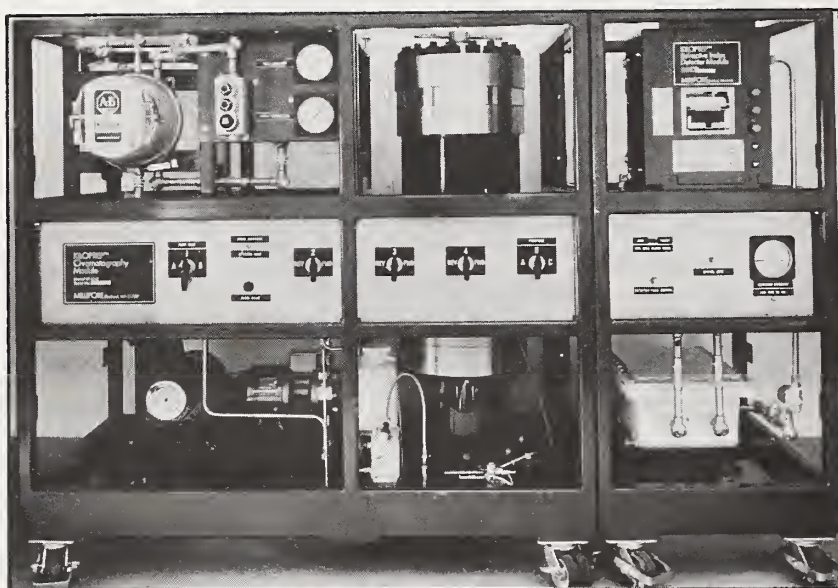


Figure 5.--Process scale chromatograph.



Figure 6.--Large scale axially compressed column.

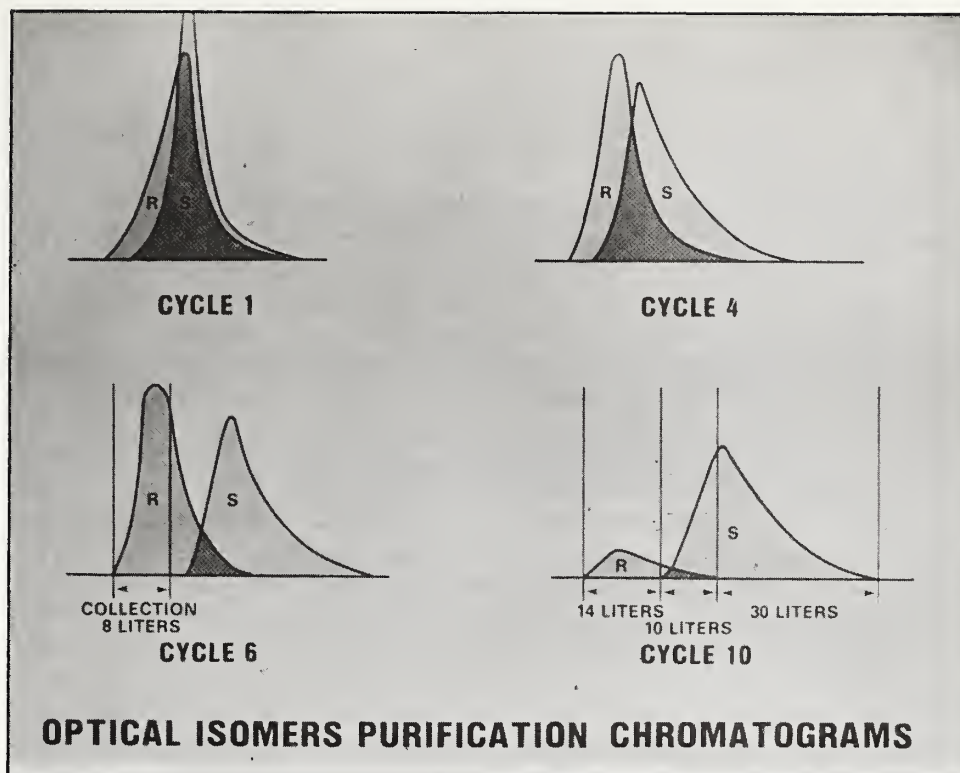


Figure 7.--Examples of purification obtained by recycling of sample on one column.

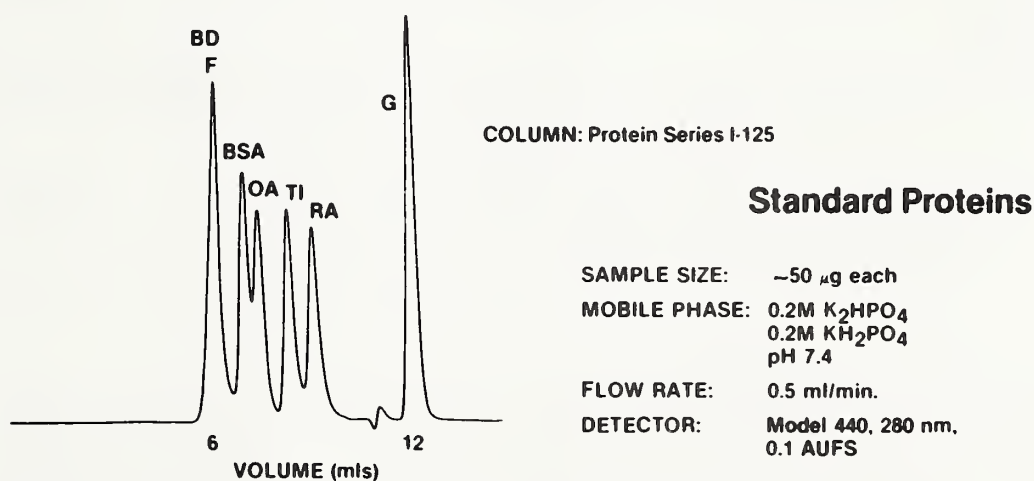
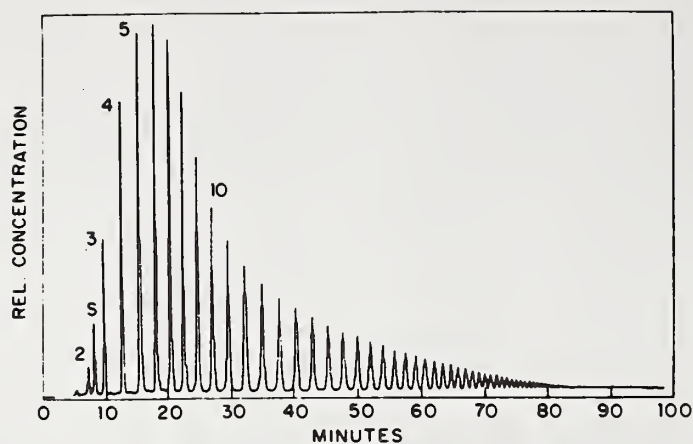


Figure 8.--Separation of proteins by size exclusion.



High-performance liquid chromatogram of a commercial poly(tetramethylene ether) glycol of molecular weight $M_n = 1000$. S is the elution time standard and 2, 3, 4, etc. are the degrees of polymerization.

Figure 9.--Absorption separation of polytetramethylene ether.

DISCUSSION

Clarke: One example of an industrial scale chromatographic process in our industry is the Finnish Sugar Co. chromatographic separation of molasses.

Hutton: Yes, sucrose recovery from molasses is certainly a good example of a large scale separation using chromatography.

Leif Ramm-Schmidt, Finnish Sugar Co.: We heard plenty about separation. You said you were going to the real big ones; you didn't tell very much about them. You stopped somewhere at a few feet in diameter. The biggest ones are used in molasses separation, for instance on a column with a diameter of 4 meters. We can desugar some 40,000 tons of molasses a year, and get out some 15,000 tons of sugar, for instance. Certainly with chromatography, we like to think of getting something else out of it. For instance, betaine is one compound that is also manufactured by us in thousands of tons a year. Then we can also get out amino acids or something else and we are doing quite a lot of work on this. In our research department for instance we have different columns starting from the ones a few inches up to, let's say, 1 meter. We also do fructose separation and xylitol. There are also some other things coming, too.

Hutton: That is certainly a very impressive scale.

Clarke: Are there any other questions on HPLC separations or on sugar analysis?

George Fawcett: I would be interested to know how many of the people here have HPLC at their disposal in their operations?

Clarke: By a show of hands, there appear to be 15 or so. There seem to be more hands up from other countries than from the U.S.--the sugar industry here has not yet become heavily involved with HPLC. Perhaps some of the remarks today will be encouraging to companies considering getting into HPLC.

This question has been presented at least twice today: How do you know that what you get off as a sucrose peak is sucrose only--that there is nothing else under the peak, and, how do you know that you get off all the sucrose that's there? Dr. Tsang did some work using radioactive labelled sugars to check on this. A known amount of C-14 labelled sucrose of known radioactivity was put over the HPLC column. The radioactivity in the sample from the section under the sucrose peak was measured. Glucose and fructose were used in similar experiments. We thus had an objective measurement of material on and off the column. We found a very good recovery of sucrose of 98%; for glucose and fructose, the recoveries were not so good, 95% for fructose, 96% for glucose.

Enrique Arias, Sugar Cane Growers Coop. of Florida: Charles, is there any work being done to try specifically to determine the polysaccharide by HPLC?

Tsang: Right now we're still in a very primitive stage because of the limitation of the R.I. detector which is not a very sensitive detector. Unless there's a considerable amount of dextran of at least 500 ppm in your sample, it won't show up on the chromatogram.

Dr. Pat Traci, Universal Foods: Mr. Ho mentioned a number like \$20-30,000 for a system. What are we talking about moneywise for dedicated in-process systems?

Ho: You mean the online system? The last time I was quoted a price around \$25,000.

Traci: So, you're not really talking about too much of a difference in money.

Ho: Actually, the basic system is there. All you have to do is spend the rest of the money in hooking up the piping which is not very much. In other words you are using the same high pressure chromatography instrument for both cases. You pay for one, you get two.

Dr. Chou, Amstar Corp.: Dr. Tsang has a very impressive list of applications, and we have one more very important one. In the northeast we have many kosher products. As you know, you are not supposed to have any corn products in kosher foods. HPLC will tell you exactly whether or not you have corn in your product. It's very important.

Clarke: That is an interesting application, where HPLC is used to differentiate corn products from cane or beet products. This same point, that you can determine corn sugars in cane or beet sugars, has been applied to some situations where invert syrup made from sucrose had been mixed and diluted with corn syrup, which is cheaper. This contamination also occurs with honey and with maple syrup. HPLC is generally used to distinguish the corn syrup that's been added as a cheap contaminant.

Hutton: Another example of this sort of use is when HPLC has been used to detect whether there have been any nonpermitted animal fats added to milk in certain circumstances. This check is for the same reason--to maintain kosher status.

Stephen Brooks: Dr. Hutton, you mentioned one of the problems a number of people having sugar analyzers do encounter. That is the problem of stability of the refractive index detector. Is there a way to improve the stability of the refractive index detector?

Hutton: I am not sure I can give a full answer to that. Thermostating is usually important, and many detectors are not thermostatted. Many of them come in large blocks of metal which are meant to act as heat sinks. These are usually bored for water circulation. I think that's a fairly important thing, because the cycling of the laboratory temperature is usually a problem. Waters Associates, at one time, made a high temperature chromatograph for gel permeation chromatography. There were particular efforts to make that R.I. detector more stable and more sensitive, and it's about tenfold more sensitive. But that detector has never made its way out into other chromatographs. The electronics for it were commingled with the rest of the complex electronics for that instrument and therefore it could never be pulled out as a unit and sold.

I think the other answer to this question is an oblique one. We are currently seeing a spate of other methods of detection. The basic problem analyzing carbohydrates was that there really was no UV absorption inherent in them, but now we have low wavelength detectors to use on carbohydrates. For a long time, it seemed as if there were very few good chemical methods for conferring absorptivity or detectability. We are now beginning to see a multiplicity of both precolumn and post column methods of derivatization, which makes them far more detectable. The first one that appeared several years ago was the tetrazolium blue reaction for reducing sugars. I think what may eventually happen is that the problem of refractive index detector sensitivity and stability may be swept away by other methods of detection.

Dr. M. Ladisch, Purdue University: We have been able to use the refractive index detector to detect glucose down to 2 parts per million. After many years of experience, we found that, first of all, you really don't want to hook up a water bath because every time the water cycles, the pen will go a full strip chart length up or down. It's like a saw tooth curve. It's a good way of testing to see if your water bath controller is doing what it is supposed to be doing. What we found was the best thing is to put it in the room and let it seek its own temperature. And that way the baseline will stabilize and over a day you might have a slight drift, but the integrator will take care of it. The other thing we found is you can change the inside diameter of the column to 20 microns. By so doing, you decrease dispersion of the peak. I think one thing Dr. Hutton usually mentions in his excellent lectures is that fact that dispersion is a big factor in chromatography. The reason that corn sweeteners aren't any cheaper than they are is the fact that when you run them through their particular large scale chromatographs, which are really big, they get a dilution from 20% syrup down to 1-2%. They have to concentrate it back up. The same occurs in analytical LC. If you inject a sample that contains 1% sugar, by the time it travels the short length of the column, it might be diluted to a fraction of a percent. By controlling the

inside diameter of the column, we found you make the peaks a lot sharper. Once you do this, you still get separation. You're able then, to use an R.I. detector down to the parts per million range. But it requires a tremendous amount of sample clean-up. I guess the real problem is that real life is dirty--it's not clean. It gets back again to the question of sample clean-up.

Clarke: Thank you. I might add there that in our own work, we have remarkably little trouble--it may just be that we've been lucky with the instruments--with refractometer stabilization. We can routinely detect 10 parts per million or so of sucrose in things like boiler feed waters, which of course are very clean. That's been our experience as far as limit goes, with an R.I. detector.

John Williams, Tate & Lyle: There is another approach that is being marketed by one company for detecting sugars. It's the company that sells ion chromatographs. And they have an electrochemical detector, with several electrodes and several pulse switchings which oxidizes the sugars and makes it more sensitive than the refractive index. I think you have to have a high pH to run it and that might be incompatible with many chromatographic conditions. Have you had any experience with that?

Hutton: That is the pulsed amperometric detector. It involves cyclic voltametry with rapidly oscillating sign of the current. This is to essentially depolarize the electrodes to get the reaction to occur. It must be operated at either pH 1 or at pH 13. One difference about this approach is that at the high pH, all the indications are that you're actually chromatographing carbohydrate anions. Whatever mode of chromatography that one uses for carbohydrates, no one seems to agree very well on what is actually going on because of the various puzzling elution orders in different systems. In this case, at the high pH end you are apparently using the anion, and at pH 1, a protonated carbohydrate cation.

